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RHIZOCTONIA FOLIAGE BLIGHT OF SUGAR BEETS[1]

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INTRODUCTION

In July 1938, after a rather prolonged period of rainy weather, a previously unrecognized disease affecting the foliage of sugar beets (*Beta vulgaris* L.) was found in experimental plantings of the Department of Agriculture at Arlington Experiment Farm, Arlington, Va. The disease occurred in the experimental plots there in the 1939, 1940, and 1941 seasons and since then each year in the experimental plantings of sugar beets at the Plant Industry Station, Beltsville, Md. It was rather difficult to find affected plants after an extended period of dry weather. Observations during the past 5 years have shown that the disease occurs also rather generally in Michigan, Illinois, Wisconsin, and Minnesota. In 1945 it was found in an irrigated field at Las Animas, Colo. High incidence and severe effects of the disease appear to be directly associated with prolonged periods of high humidity.

In a brief report (*10*) [2] foliage blight was attributed to a strain of *Rhizoctonia solani* Kühn which has a distinctly different pattern of attack from that associated with the *Rhizoctonia* strains that cause sugar-beet crown and root rot and dry rot canker. So far as the writer knows, this disease has not been described elsewhere. In 1924 Schenck (*22*), however, reported the occurrence of a *Hypochnus* that produced a cobweblike mycelium on the surface of sugar-beet leaves in Germany. Apparently this fungus was almost entirely superficial, since Schenck found no evidence of penetration of the leaf tissue and did not mention any necrotic spotting of leaves. It produced basidia chiefly on the lower side of the leaves. From a study of spore characters Schenck suggested (*22, p. 322*) that the fungus be called either *Hypochnus betae* or "*Corticium vagum* B. and C. var. *betae*," but she gave no formal description. Her inoculation experiments with potatoes (*Solanum tuberosum* L.) and sugar beets did not result in infection. This organism is of interest because of its fruiting habit, but its lack of pathogenicity makes it distinct from the strains of *Rhizoctonia solani* that cause sugar-beet foliage blight in the United States.

[1] Received for publication October 22, 1946.

[2] Italic numbers in parentheses refer to Literature Cited, p. 313.

After the discovery of sugar-beet foliage blight cultural and pathogenicity studies were made to determine the identity of the causal organism, its mode of dissemination, its range of pathogenicity, and the nature of the strains that exist within the organism. This paper reports the results of these studies.

DESCRIPTION AND PROBABLE COURSE OF FOLIAGE BLIGHT IN THE FIELD

Characteristic symptoms of foliage blight are found on sugar-beet plants that have reached the 6- to 10-leaf stage and on larger plants. The foliage-blighting *Rhizoctonia* is commonly manifested by attacks on the heart leaves. These it reduces almost to stubs consisting of the petiole with distorted portions of the leaf blade and blackened fragments of diseased tissue (fig. 1). It produces shallow, brown or

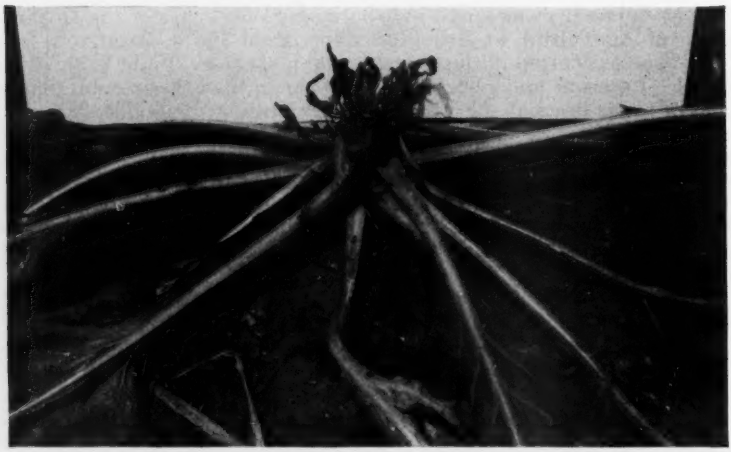

FIGURE 1.—Rhizoctonia foliage blight on the heart leaves of a sugar-beet plant, where it causes necrosis of petioles and blackened margins and tips of leaf blades. Note the shallow cankers on some of the petioles.

black cankers on the petioles, causing them to become distorted. Continued attacks may suppress or retard the growth of the heart leaves. In dense stands such blighted plants may shortly be overgrown by neighboring plants and be overlooked. Blighting effects are seldom observed on the taller leaves in the outer whorls of sugar-beet plants before July.

After a period of wet weather the disease appears on the larger leaves as irregular, blighted areas, sometimes involving a third to a half of the fully expanded leaf blade (fig. 2). For a brief period the affected areas have a water-soaked appearance. Then they become dark brown and finally black. One or more leaves of a plant may be affected. At relative humidities approaching 100 percent and with temperatures of 21° to 25° C. the disease progresses rapidly. Under less humid conditions and with lower temperatures the growth of the

causal fungus is confined to the crown region of the sugar-beet plants, where it causes necrosis of the tips and margins of the young leaves. As the weather becomes warmer, periods of high humidity set up conditions favorable for the development of the basidiospore stage of the organism. This stage, which consists of a powdery, grayish-white growth, is found almost exclusively on the ventral side of the

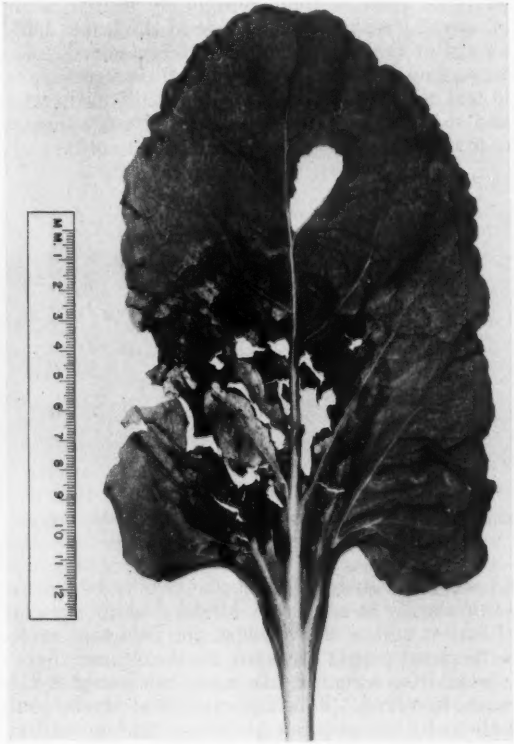

FIGURE 2.—Fully expanded leaf blade, showing large, necrotic area caused by foliage-blighting *Rhizoctonia*. Note that on drying the affected areas become brittle, fall out, and leave holes that resemble injury by hail or by certain insects.

leaves. It develops chiefly on sound tissue adjacent to necrotic areas and on sound portions of petioles adjacent to the black cankers (fig. 3). The perfect stage of the foliage blight fungus is very delicate and of rather short duration.

As indicated, the formation and release of the basidiospores are dependent upon warm, humid weather. By the basidiospores the fungus is disseminated for a considerable distance, possibly 100 feet or more. Under favorable conditions the basidiospores germinate within 12 hours and penetrate the sugar-beet leaf, where the growth

of the mycelium is very rapid. From the place of initial infection, usually a necrotic area about 5 mm. in diameter, the mycelium ramifies on the surface of the leaf and hyphal tips enter the leaf here and there through the stomata or penetrate directly after forming infection cushions. With the rapid invasion of the parenchyma several infection areas may coalesce, the area of involvement may increase, and the conspicuous leaf blight may result. The chlorophyll of the parenchyma cells is soon completely destroyed by the *Rhizoctonia*, and only the transparent cuticle of the leaf is left unaffected. The perfect stage of the fungus may again be formed, and the release of basidiospores may bring about secondary dissemination. Contact of a diseased leaf with a healthy one may result in direct infection of the latter, and some extension of the fungus from a diseased plant to its neighbors may also be brought about by the contact of leaf blades or petioles.

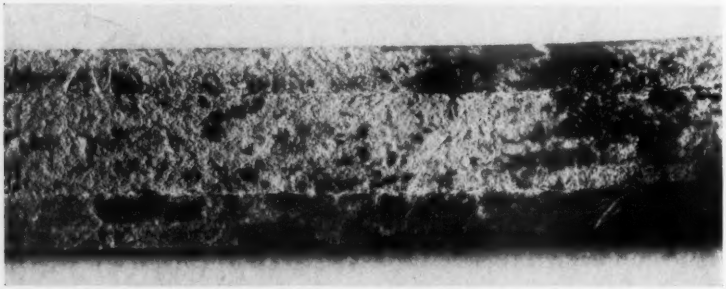

FIGURE 3.—Sugar-beet petiole, showing the basidial stage of the *Rhizoctonia* that causes foliage blight. × 6.

In the field several slightly affected plants may be found sometimes in immediate proximity to a severely blighted plant, which apparently was infected earlier and is the focus of the infection area. Usually, however, the diseased plants are more scattered and there is no clear evidence of spread from a center. In many cases only the blades of the larger leaves are involved. This indicates that the late infection was not brought about by the surface extension of the causal organism from earlier basal infections on the same plant, but by basidiospore infections. With the onset of dry weather the diseased spots on the leaves fade to a light color and become brittle. The brushing together of the leaves by the wind causes the dead areas to shatter out and leave holes that bear superficial resemblance to injury by insects or hail.

Microscopic examination of the margins of shattered-out rents in a leaf reveals *Rhizoctonia* hyphae. When a rather long humid period is followed by a short dry one, a dead central area may be surrounded by a green border on which *Rhizoctonia* threads creeping on the leaf surface are clearly visible. When a second humid period occurs, a zone or a more or less complete ring of affected tissue may appear a centimeter or more from the initial dead area, indicating a second onset of the disease. Presumably this is due to a second penetration of the leaf tissue by the fungus.

The foliage-blighting *Rhizoctonia* was found capable of causing both preemergence and postemergence damping-off of sugar-beet seedlings. In greenhouse tests damping-off has often been severe, and the fungus appears able to persist in the soil. In field plantings at Beltsville, Md., however, no serious damping-off attributable to the foliage-blighting *Rhizoctonia* has occurred. After the seedling emerges, the fungus may attack the cotyledons, the first young leaves, and the growing point, retarding growth and in some cases causing death. Attack on the growing point generally results in necrosis of the leaf tips and margins of the unfolding leaves. On further growth the injured leaves present a tipburned, blackened appearance characterized by irregularity of the leaf outline. The disease is more conspicuous on somewhat older sugar-beet plants.

PATHOGENICITY STUDIES

METHODS OF INOCULATION

The pathogenicity of numerous isolates of the *Rhizoctonia solani* associated with the foliage blight was tested under greenhouse conditions by inoculations of sugar beet, as well as of other plants including bean (*Phaseolus vulgaris* L.), alfalfa (*Medicago sativa* L.), Italian ryegrass (*Lolium multiflorum* Lam.; *L. italicum* A. Br.), bromegrass (*Bromus inermis* Leyss.), and potato. The pathogenic effects on foliage, on stand of seedlings, and on half-grown roots of sugar beets received most attention.

The inoculum for pathogenicity tests on sugar beets and the other species was obtained by making cultures of the various isolates on 8 ounces of sterilized sand-corn-meal medium (95:5) in 500-cc. wide-mouthed flasks. Usually a culture period of 2 weeks was used.

In the tests on sugar-beet and grass seedlings aliquots of sterilized compost soil, sufficient to fill three previously sterilized 6-inch flowerpots, were inoculated by adding an 8-ounce culture of each isolate. After a thorough mixing the soil was placed in the 6-inch pots and allowed to stand on rotating tables for 1 week before the seeds were planted. The utensils used in preparing the mixtures of soil and inoculum were disinfected between sets by wiping them with a piece of cheesecloth saturated with a 1:25 formaldehyde-water solution.

In the studies of damping-off of sugar beet 25 untreated seed balls were planted per pot. They were spaced half an inch apart and planted 1 inch deep. The seeding rates for alfalfa and grasses were comparable with those customarily used in the field. The seeds were sown broadcast and then worked into the surface soil with the wooden label placed in each pot.

The soil was inoculated in the same manner for the potato experiments. The seed pieces, cut from tubers previously disinfected for 30 minutes in a solution of mercuric chloride and water (1:1,000), were placed 4 inches below the surface of the soil at the time when the pots were being filled with the inoculated soil. The emergence of the sprouts essentially synchronized with the germination and emergence of the sugar-beet, alfalfa, and grass seedlings.

INOCULATION OF LEAVES, PETIOLES, AND CROWNS OF SUGAR BEET

The effect of various *Rhizoctonia* isolates on sugar-beet leaves was studied in two humidity compartments of the greenhouse. These 9- by 9-foot, glass-walled compartments communicated with the main greenhouse by a glass door on one side and were equipped on the other three sides with 30-inch greenhouse benches. Devices were installed to maintain essentially 100-percent relative humidity and to control temperatures. A water line controlled by a single valve was placed 5 feet above the benches so that water might be sprayed into the air from seven evenly spaced, atomizing nozzles. A cheesecloth housing, consisting of ceiling and back drop, was supported on a frame over the benches to screen the plants on the benches from drip or direct fall of the spray. The cheesecloth cover caught surplus water and prevented excessive accumulation of water on the leaves. Without such protection inoculum placed on the leaf would have been washed off. The saturated cheesecloth also served to maintain the relative humidity at 100 percent for a considerable period after the sprays had been turned off. A thermostatically controlled unit heater, which permitted maintaining approximately 21° C. temperature at night, was installed in each compartment. The temperature in daytime was also controlled by the unit heaters, supplemented by manual regulation of individual outside ventilators on the compartments. Small quantities of inoculum were placed on the leaf blades, on the petioles, and in soil adjacent to the roots to assure the fungus reaching the growing points and petioles. *Rhizoctonia* isolates from plant parts affected with various diseases were tested (table 1).

TABLE 1.—*Pathogenicity of isolates of Rhizoctonia from foliage blight of sugar beet and from other sources, 14 days after inoculation of sugar beets*

[Each symbol based on results of 3 replications]

Isolate	Source of isolate	Place of collection	Year of isolation	Effect[1] on—		
				Leaves	Petioles	Crowns
ISOLATES FROM FOLIAGE BLIGHT OF SUGAR BEET						
R-254	Blighted leaf	Arlington, Va.	1938	++	++	+
R-352	Blighted leaf	do	1940	++++	++	+
R-355	Blighted leaf	do	1940	+++	++	+
R-360	Blighted leaf	Doylestown, Wis.	1940	++	++	+
R-394	Blighted leaf	Beltsville, Md.	1942	++	+	+
ISOLATES FROM CROWN AND ROOT ROT OF SUGAR BEET						
R-167	Rotted root	Arlington, Va.	1934	+	+++	+++
R-216	Rotted root	Wheatland, Wyo.	1936	+	+++	+++
ISOLATES FROM POTATO						
R-72	Sclerotium on tuber.	Greeley, Colo.	1926	−	−	−
R-189	Sclerotium on tuber.	Presque Isle, Maine	1934	−	−	−
R-311	Canker on sprout	Arlington, Va.	1938	−	−	−
R-76	Spot on leaf	East Lansing, Mich.	1929	−	−	−

[1] −, No tissue involvement; +, slight spotting, spots not more than 0.5 cm.; ++, spots average more than 1 cm.; +++, spots average 2 cm. or more; ++++, third or half of leaf blighted. Inoculation with sterile medium resulted in no infection.

Under the favorable moisture and temperature conditions of the compartments, the strains of the foliage-blighting *Rhizoctonia* (R–254, R–352, R–355, R–360, and R–394), representing isolates obtained over a number of years and at various places, gave evidence of infection after 24 hours and the area of parenchyma involved was at least 1 cm. broad in 48 hours. In the first 4 days of this test the maximum extension of the fungus on a fully expanded leaf caused a necrotic area 8 cm. in diameter. This degree of tissue involvement was in decided contrast to that caused by the other *Rhizoctonia* strains included in the test, since there was no visual evidence of infection by these isolates even after 48 hours. Rapidity of infection and the rather extensive involvement of tissues distinguished the foliage-blighting type of *Rhizoctonia* from the root-rotting type obtained from sugar beet. The latter, however, may tardily cause slight infection; for example, after 14 days' exposure only limited spotting of the sugar-beet leaves at points of inoculation was noted (table 1). The four isolates from potato included for comparison (R–72, R–76, R–189, and R–311) were nonpathogenic to sugar-beet leaves. However, isolate R–76, obtained from a leaf spot on potato, was found in other tests to be mildly pathogenic. Inoculations of sugar-beet petioles with isolates from potato indicated that they were not able to invade such tissues.

The mycelium that grew from the inoculum of the isolates from foliage blight that was placed in the soil near the sugar-beet plants caused shallow, brown cankers on the petioles and distortion. The leaves, however, did not wilt. The young, emerging leaves at the growing point of the plant also were attacked and reduced to tip-burned stubs similar to those found in the field. In some cases the continued attack of the foliage-blighting *Rhizoctonia* caused the destruction of all the foliage. Upon removal to drier atmospheric conditions, however, many such plants produced new leaves largely free from spots.

The attack resulting from placing inoculum of the isolates from sugar-beet crown and root rot in and on the surface of the soil near the sugar-beet plants was noticeably different from that caused by the foliage-blighting isolates just described. Instead of forming shallow cankers, these isolates invaded the base of the petioles and caused them to blacken and to become so weakened that the leaves wilted and flattened down on the surface of the soil. The roots rotted at the crown, and when removed to drier atmospheric conditions such plants did not recover.

INOCULATION OF SUGAR-BEET SEEDLINGS

In 1941, 33 *Rhizoctonia* isolates, obtained from the foliage blight of sugar beets in different years and from several geographic locations, were tested for pathogenicity to sugar-beet seedlings. The results obtained in this test (table 2) and in many others indicate that some of the isolates that cause blighting of sugar-beet foliage are also highly pathogenic to seedlings, killing 100 percent of the inoculated plants. There is considerable variation in the pathogenicity of the several isolates; some are extremely aggressive, whereas others are only mildly pathogenic.

TABLE 2.—*Pathogenicity of 33 isolates of the Rhizoctonia from foliage blight of sugar beet as indicated by relative stands of sugar-beet seedlings 3 weeks after planting in inoculated soil, 1941*

[Each value is the average of 3 replications]

Year and place of collection and isolate	Stand as compared with that of check [1]	Year and place of collection and isolate	Stand as compared with that of check [1]
1938		*1940*—Continued	
Arlington, Va.:	*Percent*	Arlington, Va.—Con.	*Percent*
R-254	38.5	R-347	2.4
R-255	3.8	R-348	.7
R-256	9.6	R-349	.0
R-257	13.5	R-350	17.5
R-258	5.5	R-351	.7
R-259	.7	R-352	1.4
R-260	3.8	R-353	.0
R-261	23.1	R-354	8.6
R-263	6.2	Waseca, Minn.:	
R-266	36.1	R-355	.0
R-267	5.5	R-356	.0
R-268	.0	R-357	.0
R-269	.7	R-358	.0
R-270	.0	R-359	.0
1940		Doylestown, Wis.:	
R-344	1.4	R-360	36.8
R-345	.0	R-361	67.3
R-346	.0	R-362	44.0

[1] The checks in this experiment had actual stands of 38, 42, and 45 seedlings.

INOCULATION OF SUGAR-BEET ROOTS

Several experiments were conducted to determine the pathogenicity of the foliage blight *Rhizoctonia* to sugar-beet roots. In one such experiment three isolates representative of the foliage-blighting *Rhizoctonia* strains, three representative of the root-rotting types, and three from potato were tested for pathogenicity to sugar-beet roots. Field-grown roots of 3- to 6-cm. diameter were disinfected in a 1:1,000 solution of mercuric chloride for 30 minutes. The roots were then rinsed in distilled water and dried. After this treatment the roots were planted in 5-inch pots that had been sterilized and filled with sterilized sand. The taproots of half the collection were not injured before inoculation, and the inoculum was placed adjacent to the root approximately 1 inch below the surface of the sand. The remaining roots were injured by removing a plug about 1 cm. in diameter and 3 cm. long by means of a cork borer. The resulting hole was packed with inoculum held in place by a 5-mm. section of the plug forced into the opening. The roots were then planted. All pots were watered regularly with tap water and were kept on rotating tables to equalize temperature and light conditions. After 1 month the roots were removed and data were recorded. The existence of softened, discolored tissue more than 2 mm. from the wounded surface was taken as evidence that some invasion of the root had taken place.

The results from this experiment do not require tabulation, since they were very definite for the three types of *Rhizoctonia*. The foliage-blighting strains did not invade the uninjured taproots at all, and the extension of injury or decay at the borders of the hole made by removing a plug of tissue was small and probably not attributable to the inoculum. Similarly, the isolates from potato did not cause positive infection of either the uninjured or the injured roots. On

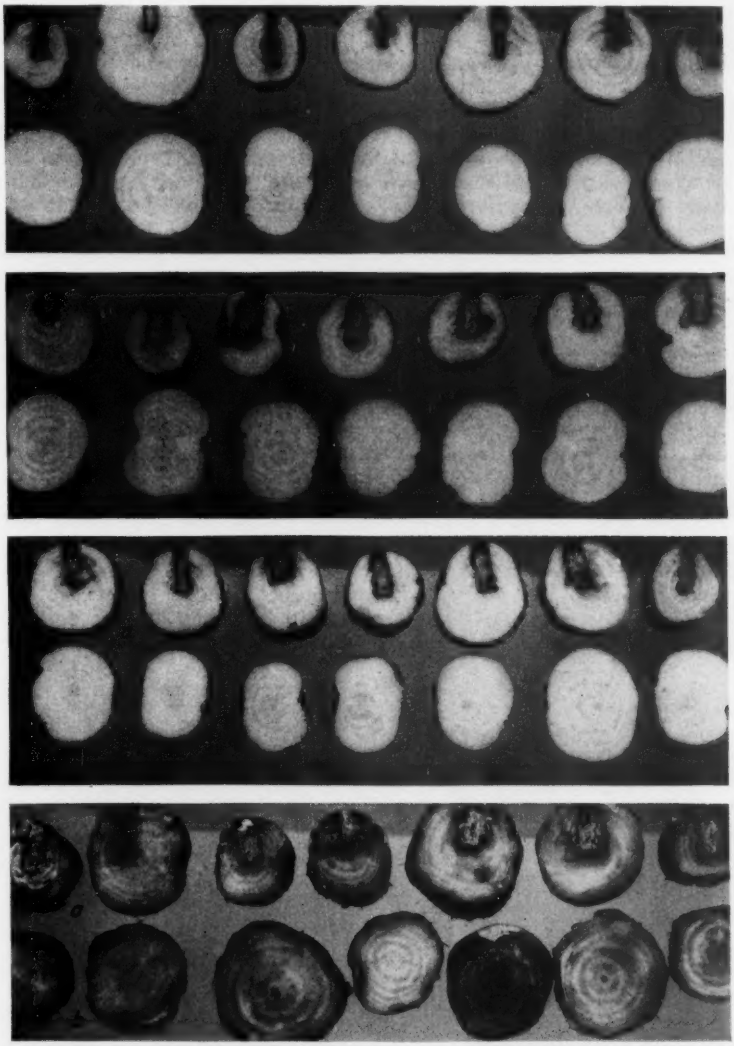

FIGURE 4.—Sections of sugar-beet roots made through the points of inoculation: *A*, No organism (check); *B*, *Rhizoctonia* isolated from sugar-beet foliage blight (R–355); *C*, *Rhizoctonia* isolated from a sclerotium on potato (R–72); *D*, *Rhizoctonia* isolated from typical sugar-beet crown and root rot (R–216). *a*, Roots injured prior to inoculation; *b*, roots not injured.

the other hand, the root-rotting types invaded the roots and caused typical rhizoctonia rot, whether the roots were wounded or not. Figure 4 illustrates the results obtained.

The pathogenicity of various *Rhizoctonia* isolates to sugar-beet roots was tested under field conditions for a number of years, and isolates of foliage-blighting *Rhizoctonia* were included from time to time. Of 53 such isolates tested, 48 were definitely nonpathogenic to sugar-beet roots. Some rotted plants were found in the plots in which the other 5 isolates were used, but these results are discounted because of occasional rotting of the adjacent check roots due to the natural occurrence of some other pathogenic *Rhizoctonia* in the field. Root rotting is, therefore, not to be expected from the *Rhizoctonia* strains that cause foliage blight, but it is not entirely precluded.

CROSS-INOCULATION OF VARIOUS SPECIES

The pathogenicity studies on species other than sugar beets were limited to Stringless Green Refugee bean, Grimm alfalfa, Italian ryegrass, bromegrass, and Green Mountain potato. The technique used is described on page 293. The tests consisted chiefly in a study of the pathogenic effects of the isolates from the sugar-beet foliage blight on the seedling or early growth of the various crop plants, but other *Rhizoctonia* isolates were included for comparison. The experiments on all crops were not carried on simultaneously but at intervals during the course of the work. For instance, bean and potato were included in one series of experiments and alfalfa, Italian ryegrass, and bromegrass in another. The results of these tests showed that the foliage blight *Rhizoctonia* is pathogenic to all of these crops. On bean, however, the variability among the foliage isolates is again shown; one isolate (R–394) proved nonpathogenic, another moderately pathogenic, and the other three severely pathogenic to this host

FIGURE 5.—Leaves of bean plants: *A*, Not inoculated (check); *B*, inoculated with a pure culture of *Rhizoctonia* from a sugar beet affected with foliage blight (R–422).

(table 3). On bean the cotyledons are attacked and deep cankers form on the stems. Blighting of the bean foliage also occurs when inoculated plants are kept under conditions of high relative humidity and high temperature (fig. 5). Alfalfa stands were reduced by the foliage blight *Rhizoctonia* to from 4.0 to 7.3 percent of the stand obtained from seed planted in sterilized soil. Retarded emergence and greatly reduced stands of Italian ryegrass and bromegrass resulted from inoculation with four isolates. Near the conclusion of the experiment one pot each of ryegrass and bromegrass for each of the isolates used was transferred to the humidity compartment (p. 294) and subjected to a misty spray. The foliage-blighting isolates attacked the blades of the grasses within 96 hours and caused their complete destruction in a week.

In the inoculation tests with potatoes the number of days elapsing from the date of planting to emergence of the sprouts, as well as the

TABLE 3.—*Pathogenicity of various isolates of Rhizoctonia from sugar-beet foliage blight in comparison with that of isolates from other hosts on bean, potato, alfalfa, Italian ryegrass, and bromegrass*

[Each value is the average of 3 replications]

Isolate	Source of isolate	Place of collection	Year of isolation	Disease produced on—				
				Bean (stand as compared with that of check)	Potato [1]	Alfalfa seedlings (stand as compared with that of check)	Italian ryegrass [1]	Bromegrass [1]
				Percent		*Percent*		
ISOLATES FROM FOLIAGE BLIGHT OF SUGAR BEET								
R-254	Blighted leaf	Arlington, Va.	1938	12.5	+			
R-352	do	do	1940	.0	+			
R-355	do	Waseca, Minn.	1940	.0	+			
R-360	do	Doylestown, Wis.	1940	50.0	+			
R-394	do	Beltsville, Md.	1942	100	+			
R-420	do	do	1943			4.0	+	+
R-421	do	do	1943			7.3	+	+
R-422	do	do	1943			5.5	+	+
R-423	do	do	1943			4.0	+	+
OTHER RHIZOCTONIA ISOLATES INCLUDED FOR COMPARISON								
R-167	Crown rot of sugar-beet roots.	Arlington, Va.	1934	0.0	+			
R-216	do	Wheatland, Wyo.	1936	.0	+	100	−	−
R-403	do	Beltsville, Md.	1942	.0	+			
R-72	Sclerotium on potato tuber.	Greeley, Colo.	1926	100	+			
R-189	do	Presque Isle, Maine.	1934	100	−			
R-301	Canker on potato sprout.	Arlington, Va.	1938	100	−			
R-76	Spot on potato leaf.	East Lansing, Mich.	1929	.0	+			
R-40	Alfalfa roots (infected).	do	1924			4.0	−	−
R-291	do	Lovell, Wyo.	1938			1.5	−	−
R-332	do	Arlington, Va.	1934			.0	+	+
R-397	do	Bard, Calif.	1942			100	−	−

[1] +, Positive infection on majority of plants exposed; −, absence of infection.

height of the plants after 2 weeks of growth, afforded very effective means of appraising the pathogenic effect of the foliage blight *Rhizoctonia* strains in comparison with those of the other *Rhizoctonia* isolates. The underground parts of the plants were examined after completion of the experiment. The examination showed that the retardation of emergence of sprouts in the foliage blight series was attributable to the attack of the fungus on the eyes of the tuber seed pieces while they were sprouting. Some growth took place, but the attack continued as the sprouts were growing through the soil. In

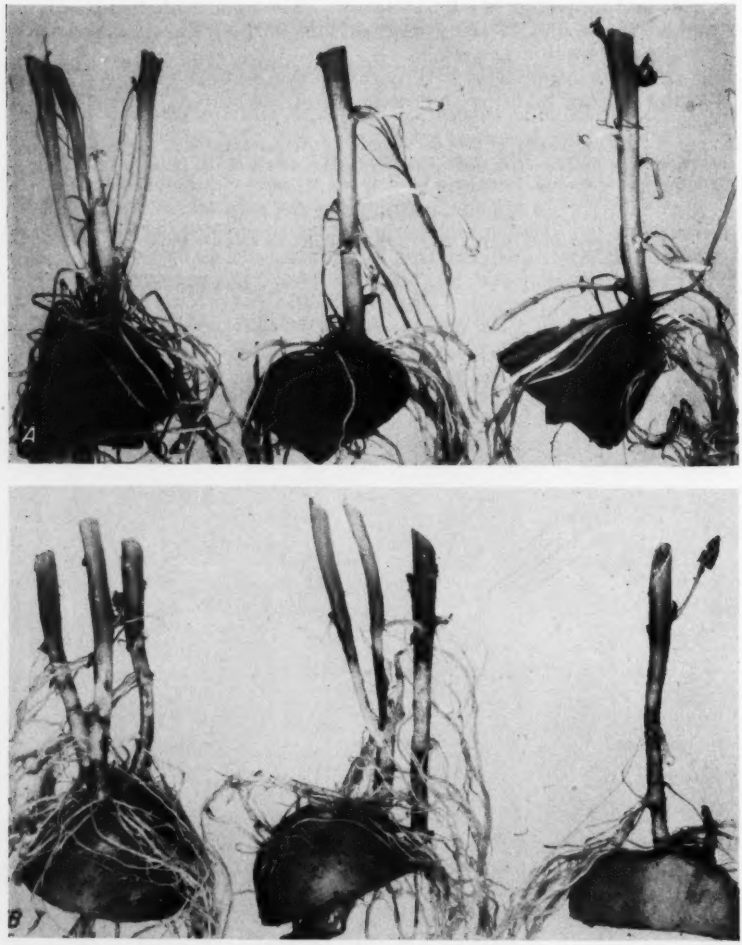

FIGURE 6.—Sprouts from potato seed pieces in soil: *A*, Containing sterile medium; *B*, inoculated with a strain of *Rhizoctonia* (R–360) isolated from sugar-beet foliage affected with blight. Note cankers on the sprouts in *B*.

some cases the growing point was injured to such an extent that a new leader developed from a lateral bud on a sprout and this in turn was attacked by the fungus (fig. 6). Both the foliage blight *Rhizoctonia* and the isolates from sugar-beet crown and root rot produced numerous cankers on the potato sprouts that grew in soils inoculated with them. However, the sprouts from seed pieces planted in soils that were inoculated with isolates obtained from sclerotia on potato tubers and from a canker on a potato sprout emerged as rapidly as the sprouts of tubers planted in uninoculated soil. It is of interest, however, that R–76, obtained from a spot on a potato leaf, very pronouncedly retarded top growth of potatoes. The sprouts were excessively cankered by this isolate.

The results of the pathogenicity tests show that, although the isolates from sugar-beet foliage blight fall into a general group, they exhibit a considerable range in pathogenicity (tables 1, 2, and 3).

THE PATHOGEN

The foliage blight of sugar beet was recognized from the beginning of the experimental work as assignable to *Rhizoctonia solani*. Investigations of the *Rhizoctonia* stage were chiefly cultural.

CULTURAL REACTIONS OF MYCELIAL ISOLATES

From affected leaves and petioles of sugar-beet plants growing in Virginia, Maryland, Wisconsin, and Minnesota, 52 isolates were obtained. The majority were from diseased plants in experimental sugar-beet plots at Arlington, Va., or at Beltsville, Md. As was to be expected from experience with *Rhizoctonia* isolates from crown rot of sugar beets, these isolates, although causing strikingly similar symptoms on sugar-beet foliage, were variable in growth habit, rate of growth, and color when grown on potato-dextrose agar in test tubes or petri dishes.

At first mycelial growth is white, but later it ranges from light yellowish brown (10 YR 6.0/3.5) (*5*, *7*, *17*) to dusky brown (10 YR 1.8/2.3). It may be dense and largely aerial. The aerial growth tends to be fluffy because of the formation of loose aggregates of hyphae more or less sclerotial in nature. Or the mycelial growth may be confined for the most part to the surface of the medium. From a study of numerous isolates having the capacity to cause typical symptoms of foliage blight, the mycelium was found to average about 6.4μ in diameter. The fungus grows very rapidly on artificial media as well as on the natural host. At room temperature the average rate of growth of some cultures on 2-percent agar containing no added nutrients, as measured under the microscope, was approximately 11μ per minute.

The sclerotia formed by the fungus on potato-dextrose agar are similar to those of the *solani* group of *Rhizoctonia* (*21*). They vary from loose-textured to compact masses and from few to many. In size also they vary, some cultures producing sclerotia as large as 1 cm. in diameter.

The darkening of the substrate due to diffusion of substances from the fungus growth varies from practically none to a medium amount giving the potato-dextrose agar a color value of dark yellowish brown

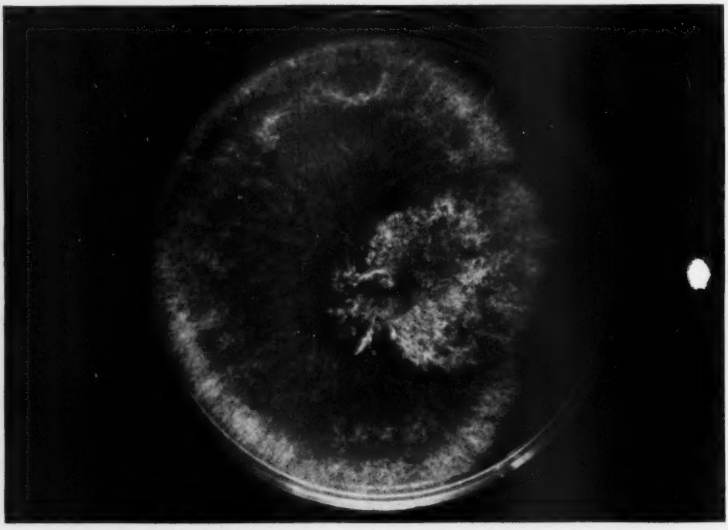

FIGURE 7.—Sector formed by a hyphal-tip isolate of the *Rhizoctonia* that causes foliage blight of sugar beets (R–361).

(0.5 YR 1.7/3.3) when viewed with transmitted light. A heavy diffusion into the substrate produces a color classified as brownish black (5 YR 0.7/0.8).

Sectors have been observed occasionally in the cultures, but these have not been studied in detail (fig. 7).

THE PERFECT STAGE

MORPHOLOGY AND SYNONYMY

The perfect stage of *Rhizoctonia solani* has been known since 1891, when Prillieux and Delacroix (*18*) described it as *Hypochnus solani*. About 10 years later Rolfs (*20*) found the perfect stage on potato stems and reported it as *Corticium vagum* Berk. and Curt. var. *solani* Burt. He trapped basidiospores by placing agar plates a short distance beneath hymenial material. These spores germinated, produced typical *Rhizoctonia* growth, and formed sclerotia (*21*). Since that time the relation of *Rhizoctonia* to a *Corticium* stage has been generally accepted. Although the perfect stage of *Rhizoctonia* has occasionally been collected from diseased plants or from soil or inert objects nearby, the appearance of this stage has been relatively infrequent compared with that of the asexual stage. Furthermore, it rarely has been found in ordinary cultures made from diseased plants (*1*, *23*). Hence, it has become almost habitual to neglect the perfect stage in phytopathological studies and to minimize or disregard its function in pathogenesis. Attention of pathologists has been directed chiefly toward *Rhizoctonia* strains as pathogens, and the perfect stage has been utilized in classification of the group species *Rhizoctonia solani* and related strains.

The fungus causing foliage blight of sugar beet is distinctive among the strains of *Rhizoctonia solani* in that the basidiospore stage is commonly found in the field whenever the disease is prevalent. The hymenial stage of the foliage blight *Rhizoctonia* consists of loose wefts of mycelium that arise from vegetative hyphae through repeated right-angled branching. Globular or oblong cells much shorter than the vegetative cells are produced, and these in turn may form one or more branches. The latter may become basidia or they may continue branching. Infrequently a basidium may form directly from a vegetative cell. Four knoblike projections arise from the basidium and become tapered sterigmata. A basidiospore forms at the tip of each elongated sterigma; when the spore is mature, its longest diameter approximates the length of the sterigma. When the mature basidiospores are discharged, the sterigmata remain as sharp-pointed projections from the basidium. The basidiospores range from 8.03μ by 4.82μ to 12.86μ by 8.03μ. The average of 200 measurements of spores from field collections and of those produced by isolates under humid conditions in the greenhouse was 9.92μ by 6.97μ; no collection or isolate deviated enough from this mean to warrant particular attention.

Recently Rogers (*19*) proposed the name *Pellicularia filamentosa* (Pat.) Rogers to include the organisms previously classed (*20*) as *Corticium vagum* var. *solani*. The size and other characters of the spores would permit the classification of the fungus causing foliage blight as *P. filamentosa*.

Methods for Obtaining the Perfect Stage of Various Strains of Rhizoctonia

Briton-Jones (*2*) and Müller (*16*) observed the formation of the perfect stage of *Rhizoctonia* strains after infection of the hosts. Müller used single-basidiospore cultures for making inoculations. In general, occurrence of the perfect stage was found to be sporadic and not subject to control.

A number of isolates of the foliage blight fungus produced the perfect stage on the leaves and petioles of sugar-beet plants kept under favorable conditions in the greenhouse (approximately 100 percent relative humidity and temperatures between 21° and 25° C.). For comparison a number of other *Rhizoctonia* isolates from various hosts were tested on sugar-beet roots and leaves to determine whether any of them could be induced to form the perfect stage. Sugar-beet plants growing in sterilized soil in pots and having roots approximately 2 cm. in diameter were placed in the humidity compartments described on page 294. The roots and leaves of the plants were inoculated with vigorously growing cultures of a number of hyphal-tip isolates of *Rhizoctonia*. After approximately 2 weeks the hymenial stage was detected on the petioles and leaves of the sugar-beet plants that had been inoculated with *Rhizoctonia* isolates from alfalfa, black locust (*Robinia pseudoacacia* L.), cotton (*Gossypium hirsutum* L.), horseradish (*Armoracia rusticana* Gaertn., Mey., and Scherb.), rubber (*Hevea brasiliensis* (H. B. K.) Muell. Arg.), sugar beet, stock (*Matthiola incana* R. Br.), strawberry (*Fragaria virginiana* Duchesne), sweetclover (*Melilotus alba* Desr.), and tobacco (*Nicotiana tabacum* L.).

Of this work only that concerning *Rhizoctonia* isolates attacking cotton and rubber has been reported (*11, 12*). The study indicates that many hyphal-tip isolates of *Rhizoctonia* can be induced to form the perfect stage on living plants under more or less controlled conditions. Of the factors involved, moisture and temperature seem to be very important.

Müller (*15*) was the first investigator to report even meager success in obtaining the perfect stage of *Rhizoctonia solani* on artificial media. He grew the fungus on a rich medium for 17 days and then transferred portions of the mycelial growth to wet filter paper in small flasks. Basidiospores formed on the filter paper in two of the cultures. Müller was not able, however, to duplicate his results. Ullstrup (*25*) observed the formation of the perfect stage of *R. solani* as a result of plating sections of affected cotton seedlings on tap-water agar. He made transfers of mycelial, monosporous, and multisporous material to tap-water agar containing pieces of healthy cotton seedlings that had been surface-sterilized. Only the transfers of multisporous material again produced the perfect stage. Exner and Chilton (*3*) obtained the perfect stage of *R. solani* by growing certain tissue cultures on potato-dextrose broth, rinsing them in distilled water, and then transferring them to flasks containing rooted cuttings of alligatorweed (*Alternanthera philoxeroides* (Mart.) Griseb.). The perfect stage formed on the stems of the alligatorweed. Exner and Chilton reported failure under these conditions to obtain the perfect stage from single-basidiospore cultures.

Kotila (*9*), on the other hand, found that the single-basidiospore isolates of *Corticium praticola* formed the perfect stage on the artificial media used without the addition of plant tissue. The mass culture from which these single-spore cultures were obtained formed the perfect stage on artificial culture media and on alfalfa when it was placed under suitable environmental conditions. Since this report was made, six additional hyphal-tip isolates of *Rhizoctonia solani* from alfalfa and sugar beet that formed the perfect stage in petri dishes on 2-percent distilled-water agar have been obtained. It has also been possible to induce formation of the perfect stage by some *Rhizoctonia* cultures on the walls of flasks containing small quantities of dilute Coons' synthetic solution by following the Klebs' (*8*) principle of first obtaining vigorous vegetative growth of the fungus and then transferring to a less nutritive substrate.

Behavior of Basidiospore Cultures of Foliage Blight Fungus

During the course of the investigations of rhizoctonia foliage blight of sugar beet a study was made of a number of single-basidiospore isolates obtained from *Pellicularia filamentosa*. The isolations were made by distributing with a capillary pipette (*6*) highly dilute suspensions of basidiospores on the surface of hardened plain agar in petri dishes. Forty-eight hours later the plates were examined and single basidiospores that had germinated were located and marked by means of a fine-wire ring attached to an objective of the microscope. The marked spores were transferred to hardened plain agar in other petri dishes and examined under the microscope again to be certain that only 1 spore was present; then the spore was transferred to a test

tube of slanted potato-dextrose agar. Fifty-one germinated single basidiospores from the perfect stage produced on a sugar-beet plant inoculated with isolate R–423, a typical foliage blight *Rhizoctonia*, were transplanted in this manner. Of these cultures, 38 failed to continue growth. The other 13 grew, and macroscopic differences in growth habit soon became evident. Their characteristics were also different from those of isolate R–423. Transfers were made to hardened potato-dextrose agar in petri dishes, and after a week transfers of equal portions of each culture were made to a second series of petri dishes containing the same kind of medium. Three transfers were made from each culture. The growth produced in each of the 3 petri dishes from a given culture was strikingly similar, but there were definite differences in growth habit among sets that traced back to different basidiospores.

The cultures obtained from single basidiospores were somewhat slow in starting to grow, but once established they grew rapidly and apparently almost as vigorously as those from mycelial transfers. In general appearance a series of basidiospore cultures was indistinguishable from a series of mycelial cultures of *Rhizoctonia*. The different basidiospore isolates showed ranges in color, in degrees of discoloration of medium, of zonation, and of sclerotial formation, and in other characteristics that paralleled those usually noticed in *Rhizoctonia* cultures (figs. 8 and 9). Almost any characteristic selected from mycelial cultures could be matched by a similar one in one basidiospore culture or another. In other words, the basidiospore cultures exhibited about the same range of growth characters and had about the same general appearance in culture as would be found in a series of mycelial isolates.

Infection by Basidiospores of Foliage Blight Fungus

The important role that basidiospores may play in the dissemination of the rhizoctonia foliage blight of sugar beets, referred to in the discussion of the probable course of the disease in the field (p. 291), was clearly indicated by the results of several experiments conducted in the two humidity compartments in a large unit of the greenhouse described on page 294.

The design of the experiments was such that healthy plants could be exposed to air-borne basidiospores of the foliage blight *Rhizoctonia*, but the plants were so placed that infection by direct contact was avoided. Greenhouse-grown sugar-beet seedlings 3 to 4 inches tall were used as trap plants for the basidiospores. In 1 test 22 plants, growing in 6-inch pots of sterilized soil and designated as group 1, were placed with uniform spacings on the side benches of each of the 2 compartments. One week later 26 additional sugar-beet seedlings, growing in 3-inch pots and designated as group 2, were interspersed in each compartment among those of the first group. During the first week of the experiment the basidiospore source was a few leaves which had been collected in a sugar-beet field at Beltsville, Md., and on which the fungus was fruiting. The leaves, with the basidial-stage surface faced downward, were laid on hardware-cloth screens 18 inches above the plants, 1 being placed over each bench in 1 of the compartments. This basidiospore source was augmented later by 2 sugar-beet plants

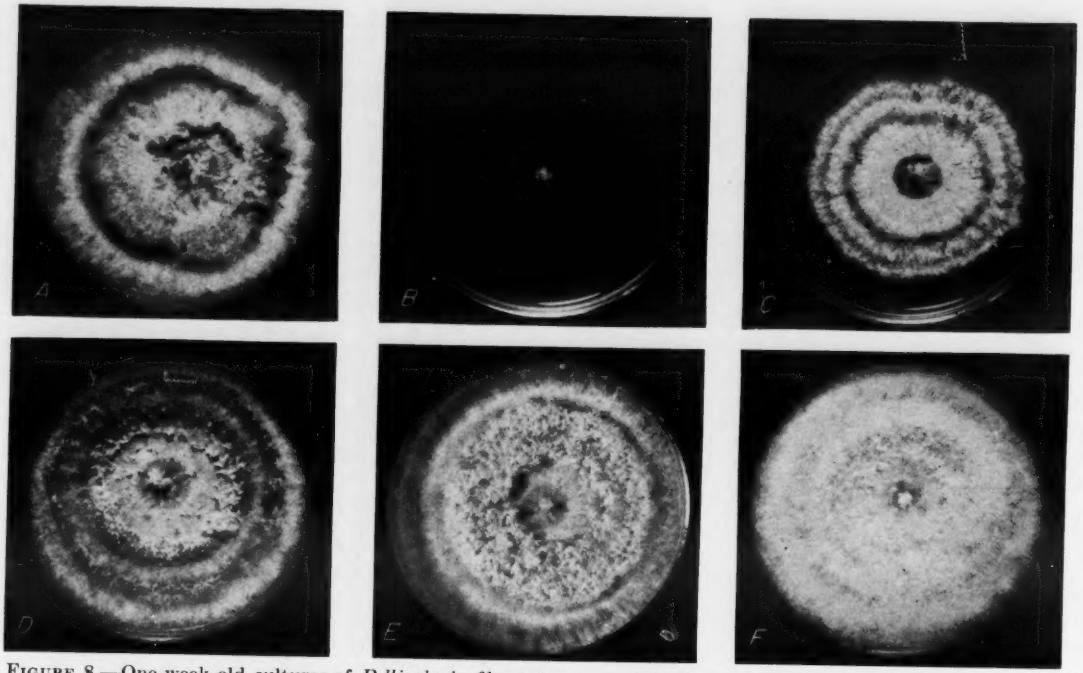

FIGURE 8.—One-week-old cultures of *Pellicularia filamentosa* on potato-dextrose agar as viewed from above: *A*, Parent mycelial culture; *B–F*, single-basidiospore cultures.

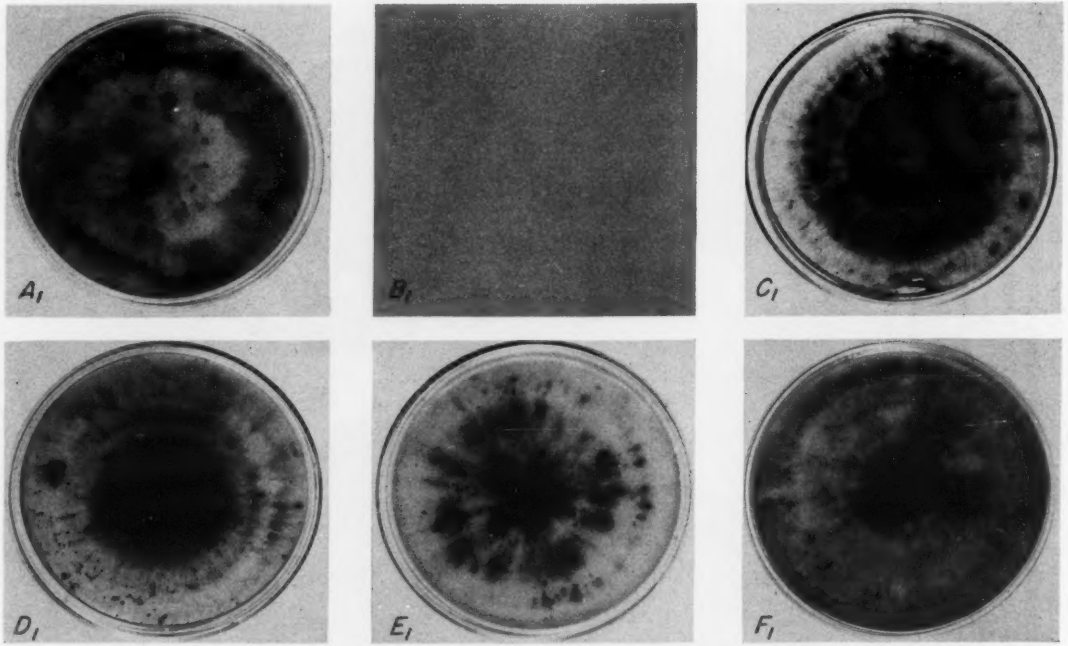

FIGURE 9.—One-month-old cultures of *Pellicularia filamentosa* as viewed through bottom of petri dish; A_1, Parent mycelial culture; C_1–F_1, single-basidiospore cultures. In this figure C_1–F_1, respectively, correspond to *C*–*F* in figure 8; the culture shown in *B* of that figure was lost.

on which the perfect stage had formed in a previous experiment. One of the plants was placed at the same level as the screens, and the other was placed on the bench 12 inches from the nearest healthy plant. In each compartment the sprays were kept on continuously for 120 hours and then turned off and on alternately to keep the humidity high.

Ten days after the start of the experiment 16 of the group 1 plants in compartment 1 showed infection. Initial infection from basidiospores appeared as minute, sunken, water-soaked, round or irregular areas. After the affected areas had reached 5 mm. in diameter, the rate of involvement of the leaf blade was greatly increased (fig. 10).

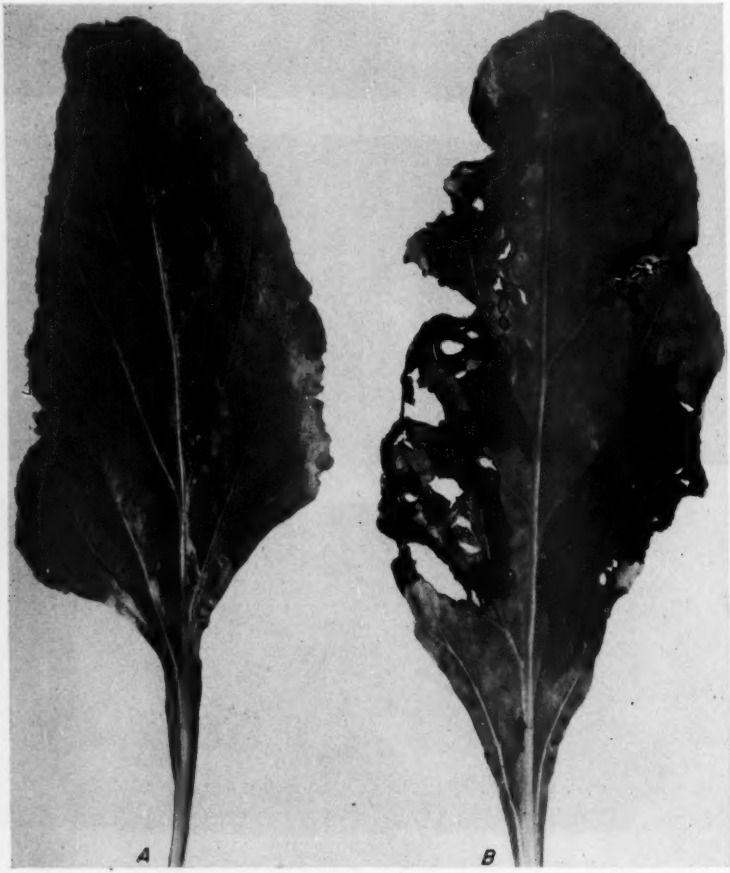

FIGURE 10.— *A*, Leaf from a sugar-beet plant grown for 14 days without inoculation in a humidity compartment in the greenhouse; *B*, leaf from a plant in a similar compartment exposed to air-borne basidiospores of *Pellicularia filamentosa*.

Microscopic examination of a number of the areas showed *Rhizoctonia* mycelium to be present. Fourteen days after the beginning of the test the 22 plants were examined in detail and the number of leaves affected and the number of distinctly separated infections brought about by the basidiospores were noted (table 4). The 22 plants in compartment 2 were all free from injury.

The final records on the experiment were taken after the group 1 plants in compartment 1 had been exposed to basidiospore infection for 24 days and the group 2 plants in the same compartment for 17 days. At this time 90.9 percent of the plants in group 1 and 76.9 percent of those in group 2 showed one or more infected areas.

TABLE 4.—*Artificial infection of sugar-beet leaves by air-borne basidiospores of Pellicularia filamentosa after 14 days in a humidity compartment in a greenhouse*

Plant and compartment	Leaves infected	Areas infected	Plant and compartment	Leaves infected	Areas infected
Compartment 1:	*Number*	*Number*	Compartment 2:	*Number*	*Number*
1	0	0	23	0	0
2	4	30	24	0	0
3	1	2	25	0	0
4	0	0	26	0	0
5	0	0	27	0	0
6	2	16	28	0	0
7	2	15	29	0	0
8	2	23	30	0	0
9	2	30	31	0	0
10	4	47	32	0	0
11	2	17	33	0	0
12	2	17	34	0	0
13	2	4	35	0	0
14	1	3	36	0	0
15	2	15	37	0	0
16	2	19	38	0	0
17	2	20	39	0	0
18	2	17	40	0	0
19	2	26	41	0	0
20	3	20	42	0	0
21	2	20	43	0	0
22	3	10	44	0	0
Total	42	351	Total	0	0

Compartment 2, which was adjacent to compartment 1 and exposed to similar environmental conditions, contained the same number of plants, but the plants were not exposed to an immediate source of basidiospores. None of the plants of group 2 became infected, and all except one of group 1 remained free from the disease. The one infected plant had a small diseased area on one leaf, and the causal organism when isolated and tested was found to be typical of the foliage blight *Rhizoctonia*. The infection is believed to have resulted from chance air dissemination of a basidiospore from compartment 1.

The results obtained in these experiments are very striking and confirm the observations made in the field which indicated that the spread of rhizoctonia foliage blight of sugar beet is brought about principally through the dissemination of basidiospores.

The pathogenicity of the 13 single-basidiospore cultures described on page 305 was determined by inoculating sterilized soil and planting untreated sugar-beet seed balls in the manner previously described and by inoculating leaves of older sugar-beet plants kept under high-humidity and high-temperature conditions. Differences in pathogenicity were noticeable in both tests, but the counts of seedling

stands afford the better numerical measurement of these differences (table 5). The single-spore isolates 3 and 5, which traced to R–423 and had the slowest rates of growth on potato-dextrose agar, were the least pathogenic of the single-spore isolates; they caused the least damping-off of sugar-beet seedlings and the least foliage infection on older plants. The other single-basidiospore cultures reproduced typical foliage blight.

TABLE 5.—*Pathogenicity of parent and single-basidiospore cultures of Pellicularia filamentosa as indicated by relative stands of sugar-beet seedlings 3 weeks after the planting of 25 seed balls in inoculated, sterilized compost soil*

[Each value is the average of 3 replications]

Isolate	Stand as compared with that of check		Isolate	Stand as compared with that of check	
	Test 1 [1]	Test 2 [2]		Test 1 [1]	Test 2 [2]
	Percent	*Percent*		*Percent*	*Percent*
R–423 (parent source)	7.7	19.1	S–17	25.8	27.3
S–3	96.4	100.9	S–27	29.2	24.2
S–5	71.6	62.7	S–33	.0	.9
S–6	18.1	20.7	S–38	13.7	17.3
S–7	7.0	7.0	S–43	7.7	16.1
S–11	15.5	23.3	S–44	3.4	14.2
S–13	44.7	38.5	S–47	10.3	12.1

[1] An average of 38.7 seedlings in the check. [2] An average of 33.0 seedlings in the check.

DISCUSSION

In this paper a new type of disease of sugar beet assignable to *Rhizoctonia solani* is reported. In addition to crown and root rot and dry rot canker previously recognized as forms of *Rhizoctonia* attack, a foliage blight with complete absence of root complications is now known. The perfect stage of the sugar-beet foliage-blighting *Rhizoctonia* occurs under the warm, humid conditions necessary for maximum disease manifestation on sugar-beet leaves. The size of basidiospores and other characters of the causal fungus permit its assignment to *Pellicularia filamentosa*. The chief emphasis in the investigations reported was placed on utilizing information obtained from basidiospore cultures to throw light on the well-known variability exhibited by *Rhizoctonia* isolates.

Previous studies showed that isolates of *Rhizoctonia* were extremely variable in pathogenicity and that there may be some trends toward group reactions. LeClerg (*13*) tested 116 isolates obtained chiefly from sclerotia formed on potato tubers and from the lesions on stems of older potato plants. Among these isolates he did not find any that were pathogenic to half-grown or mature roots of sugar beet. On the other hand, *Rhizoctonia* isolates obtained from sugar beet were pathogenic to potato.

More recently Houston (*4*) reviewed previous work and made additional studies on 260 isolates of *Rhizoctonia* (*Corticium solani*) from 15 different crop plants. He classified the isolates into cultural types. Differences in growth habit on nutrient media such as presence or absence of stroma, nature, size, and abundance of sclerotia, and absence or presence of diffusible substances that darken the medium were criteria for differentiation. Although admitting intergrades,

Houston stated that most isolates could readily be assigned to their appropriate types. Type A was obtained from many hosts and was a polyphagous type, whereas types B and C were highly specific as to hosts. Type C was essentially nonpathogenic to sugar-beet roots. Houston stated that the variation in pathogenicity among isolates was much greater when the isolates were grouped according to their original hosts than when they were grouped on the basis of cultural type. Houston's tabular data, as the author himself pointed out, revealed a number of contradictions in the proposed classification scheme as it related to pathogenicity.

In the present study it was found that the *Rhizoctonia* isolates obtained from the foliage blight of sugar beet constitute a recognizable group. This group is distinct from the isolates previously obtained from sugar beet and other hosts in that a new and previously unrecognized disease condition in sugar beets is produced. If Houston's criteria for growth habit are applied, the isolates would fall more or less into types A, B, and C; but the absence of capacity to cause root rot of sugar beet would seem to correspond only to type C of Houston's classification. Furthermore, the isolates obtained from single basidiospores of this group also showed such a great diversity of growth habit that they could be distributed among Houston's types A, B, and C. These single-basidiospore cultures also manifested great variability in pathogenicity to sugar-beet seedlings and to sugar-beet foliage.

A logical explanation for the diversity that has so commonly been found among *Rhizoctonia* isolates is afforded by the study of these single-basidiospore cultures, presumptively haploid in character. The results of the studies of the basidiospore cultures also explain why attempts to classify asexual forms on the basis of cultural characters or pathogenicity tests must be unsatisfactory.

It is recognized that the heritable factors in fungi, as in other plants, are transmitted through the nuclei. Literature on the subject was summarized by Lindegren (*14*), who suggested a rational explanation of certain phenomena on the basis of nuclear behavior. As a result of preliminary studies Exner and Chilton (*3*) stated that the fusion nucleus of *Rhizoctonia solani* is heterozygous. Müller (*16*) offered evidence that the mycelial cells of *Rhizoctonia* are multinucleate. When the reproductive stage is induced, the hymenial cells become binucleate and maintain this condition throughout hymenial growth. Fusion of the two nuclei takes place in the basidium and is followed by meiosis, after which each of the four daughter nuclei finds its way through a sterigma into a basidiospore. This is the general pattern for the Hymenomycetes and each nucleus carries a haploid complement of heritable factors. Skolko (*24*) studied the nuclear behavior in a closely related form, *Aleurodiscus canadensis* Skolko. The evidence of homothallism in spores produced on two-spored basidia and in some spores produced on three-spored basidia indicates that if four-spored basidia develop, the basidiospores on them are normally haploid.

The great divergence in growth characters and the variability in pathogenicity found among the single-basidiospore cultures tracing to individual isolates of *Pellicularia filamentosa* may be attributed to gene segregations. These take place in the basidium as a result of the reduction division that follows sexual fusion of nuclei unlike in

genic make-up. The studies of these single-basidiospore isolates indicate that *Rhizoctonia* mycelia as commonly found in nature are not homokaryotic but are heterokaryotic; that is, they are composites insofar as nuclear complements are concerned. A study of the asexual stage is, therefore, a phenotypic study interpretable only by reference to the genotypes.

It is to be noted that growth characters and the pathogenicity reactions of both basidiospore isolates and mycelial isolates are essentially parallel in character. Hence, it is concluded that growth characters, pathogenicity, and other reactions of an individual *Rhizoctonia* isolate obtained by culturing a single-hypha cell are expressions of particular assortments of genes from the nuclei that happen to be present in the cell. The reactions shown by a given culture represent the equilibrium achieved. The great multiplicity of strains among *Rhizoctonia* isolates is, therefore, related to the heterokaryotic nature of the mycelium and to the nuclear and genic assortments possible.

SUMMARY

The blight of sugar-beet foliage described in this paper has been found in Virginia, Maryland, Michigan, Illinois, Wisconsin, Minnesota, and Colorado. The conditions for its maximum development include high humidity and temperatures between 21° and 25° C. The causal organism is a type of *Rhizoctonia solani*. It attacks the small, unfolding leaves of the sugar beet and may reduce them almost to stubs. It causes cankers on petioles and attacks the blades of larger leaves. This *Rhizoctonia*, which is primarily a pathogen affecting sugar-beet foliage, does not rot half-mature or older roots. It may cause both preemergence and postemergence damping-off of sugar-beet seedlings. It is pathogenic to bean, alfalfa, bromegrass, Italian ryegrass, and potato.

The various mycelial isolates exhibited a range in growth habit, color, and rate of growth on artificial media; they varied also in pathogenicity.

The perfect stage of this *Rhizoctonia* occurs on leaf blades and petioles of sugar-beet plants. The size of the basidiospore and other characters permit its classification as *Pellicularia filamentosa*. The perfect stage was induced by growing inoculated sugar-beet plants in greenhouse compartments in which the relative humidity was kept as near to 100 percent as possible and temperatures were held between 21° and 25° C.

Field observations indicated that basidiospores play an important role in dissemination of the fungus. Experiments conducted under controlled conditions in the greenhouse confirmed these observations.

The single-basidiospore isolates were found to have cultural characters distinctly different from those of the mycelial isolate from which they were obtained. They also differed among themselves in growth habit. They varied greatly in pathogenicity to sugar-beet seedlings, infection ranging from 100 percent to almost none; on sugar-beet foliage infection ranged from mild to severe. The variations displayed by the single-basidiospore cultures are interpreted as the result of segregation of heritable characters in these haploids. The nuclear

or genic assortments that make up the heterokaryotic mycelium of the *Rhizoctonia* strains found in nature explain the multiplicity of strains that occurs within this group.

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EFFECT OF SITE ON GROWTH OF SHIPMAST LOCUST[1]

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INTRODUCTION

Shipmast locust (*Robina pseudoacacia* L. var. *rectissima* Raber) is one of several newly selected forms of black locust that have above-average growth characteristics. A number of studies on shipment locust have been published (*4*, *5*, *7*, *8*, *9*, *10*, *13*, *14*, *15*, *18*).[3] These have shown that on suitable sites, shipmast locust is straighter, more erect, and narrower crowned than the common black locust. The heartwood is definitely more durable and the trees may be more resistant to borer injury. However, observations on Long Island, N. Y., the principal area where old stands now occur, indicate that shipmast locust is particularly sensitive to site conditions. On favorable sites, magnificent stands of tall, well-formed trees occur, far surpassing in quality the common black locust. On other sites, growth of the shipmast locust is apparently much poorer than that of the common black locust.

In view of the degree to which the growth form of shipmast locust varies according to environmental conditions, its superiority over common black locust depends on the selection of sites to which it is adapted. Hence, planters cannot expect to obtain the advantages of the superior genetic characters of shipmast locust unless they have sufficient knowledge to choose satisfactory sites. Data relative to site relationships in established stands of shipmast locust on Long Island are given in the following study. These stands are the best source of information available at present on the site requirements of this variety.

METHODS

Eighty shipmast locust stands over the geographic range of the variety on Long Island were chosen for the study. The stands were so selected as to cover a wide variation in site condition, growth rate, and age. All the stands were second growth from old plantations on farms and estates.

In each of the stands usually five trees were selected in the immediate vicinity of the point chosen for soil observations. Except for the avoidance of decadent and badly damaged individuals, the trees to be measured in each stand were selected at random in respect to form,

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[3] Italic numbers in parentheses refer to Literature Cited, p. 327.

age, and growth rate. For each of the sample trees, the total age, height, and diameter breast high were determined.

The relative growth rate for each tree was indexed by means of a site-index chart (fig. 1) which was constructed according to the method

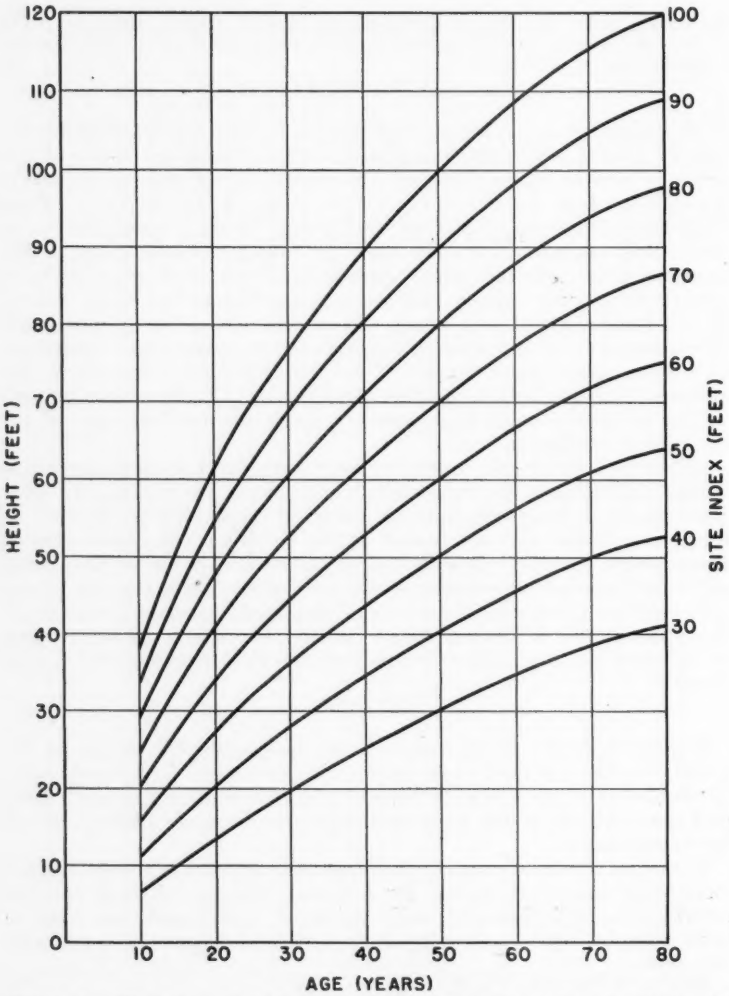

FIGURE 1.—Site-index chart for shipmast locust on Long Island. Basis: 397 shipmast locust trees, 7 to 78 years old.

described by Bruce and Schumacher (*3, pp. 318–322*). The site indexes for the trees in each stand were averaged to obtain the site index of the stand.

The site factors included in this study are listed in the first column of table 1.

The depth to water table was measured by three methods:

(1) When a dug well was present, the depth to the water table was measured with a weighted steel tape.

(2) When exposed surface water was present, its elevation in relation to the station was measured with a hand level and tape.

(3) When neither a dug well nor exposed water was present in the immediate vicinity, the water table was determined from the published water table surveys of the State of New York (*17*) and unpublished data made available by the Department of Water Supply, Gas and Electricity of New York City. These surveys were used only for stations located in areas where the permanent water table was close to the surface and the geological structure of the subsoil indicated that perched water tables were absent.

Water-table data were available for 56 of the 80 stands. The soil examination was made in a pit dug to a depth of 3 feet, from which samples were taken in each horizon for physical and chemical analyses. The standard methods of physical analysis and rapid tests (*19*) for chemical analysis were used.

In each stand a one-tenth-acre plot was laid out and a complete tally was made of all trees. Undergrowth and ground cover on the plots were recorded by species and the approximate percentage of the area occupied by each species was estimated by eye.

RESULTS

SITE FACTORS AFFECTING GROWTH RATE OF SHIPMAST LOCUST

The average site index for 56 stands of shipmast locust was 66 feet. This means that on the average, shipmast locust had been growing at the rate of 66 feet in 50 years. The slowest-growing stand showed a site index of 47 feet and the fastest-growing stand 88 feet. The

TABLE 1.—*Statistical evaluation of 39 individual site factors for indexing growth rate of 56 shipmast locust stands*

Site character	Range	Variance ratio	Site index means or regressions for significant site factors
Elevation above sea level [1]	0-250 feet	0.06	
Exposure [1]	Cool (N., NE., E., NW.) / Warm (SE., S., SW., W., flat)	5.42*	Cool=71.0. Warm=64.5.
Slope [1]	0-35 percent	5.47*	64.0+.27×slope percent.
Topography [1]	Hillsides—17 stands; flat terrain—39 stands.	27.60**	Hillsides=74.0. Flat terrain=62.5.
Depth of water table [1]	4-135 feet	1.68	
Soil series [2]	Plymouth / Haven / Sassafras / Dukes	3.49*	Plymouth=70.5. Haven=67.2. Sassafras=62.3. Dukes=60.4.
Depth of A horizon [1]	1-7 inches	.69	
Depth of B horizon [1]	5-24 inches	.06	
Depth to C horizon [1]	7-31 inches	.21	
Sand, B horizon [3]	41-95 percent	2.02	
Sand, C horizon [3]	35-97 percent	2.80	
Clay, B horizon [3]	3-16 percent	.94	
Clay, C horizon [3]	3-28 percent	.43	
Pore space, B horizon [3]	36-66 percent	3.42	
Pore space, C horizon	28-57 percent	.13	
Water-holding capacity, B horizon.[3]	16-92 percent	.13	
Water-holding capacity, C horizon.[1]	13-78 percent	1.72	

See footnotes at end of table.

TABLE I.—*Statistical evaluation of 39 individual site factors for indexing growth rate of 56 shipmast locust stands*—Continued

Site character	Range	Variance ratio	Site index means or regressions for significant site factors
Chemicals in B horizon: [3]			
Organic matter	0.3–5.1 percent	.12	
Hydrogen-ion concentration	4.0–7.1 pH	.42	
Calcium	0–4,000 pounds per acre	.12	
Magnesium	0–200 pounds per acre	.48	
Aluminum	0–500 pounds per acre	.45	
Iron (ferric)	0–300 pounds per acre	0	
Nitrogen (nitrate)	0–100 pounds per acre	.07	
Nitrogen (ammonia)	0–25 pounds per acre	.07	
Phosphorus	0–50 pounds per acre	1.32	
Potassium	0–700 pounds per acre	.05	
Manganese	0–50 pounds per acre	1.64	
Chemicals in C horizon: [3]			
Organic matter	0.0–2.8 percent	.19	
Hydrogen-ion concentration	4.3–7.1 pH	.18	
Calcium	0–3,000 pounds per acre	.23	
Magnesium	0–200 pounds per acre	.25	
Aluminum	25–500 pounds per acre	.69	
Iron (ferric)	0–100 pounds per acre	.61	
Nitrogen (nitrate)	0–100 pounds per acre	1.64	
Nitrogen (ammonia)	0–15 pounds per acre	.37	
Phosphorus	0–50 pounds per acre	4.10*	67.4–0.28 pounds per acre.
Potassium	0–500 pounds per acre	.77	
Manganese	0–50 pounds per acre	0	

*Significant at 5 percent level.
**Significant at 1 percent level.
[1] 56 stands. Variance ratio based on 1 and 54 degrees of freedom.
[2] 56 stands. Variance ratio based on 3 and 52 degrees of freedom.
[3] 55 stands. Variance ratio based on 1 and 53 degrees of freedom.

effectiveness of 39 different site factors for explaining this variation in growth rate is summarized in table 1. The second column shows the range of variation for each factor. The range is important for indicating the possible application of the results to areas outside Long Island.

The variance ratio, shown in column 3, is the customary significance test used in statistical analysis (*16*). The variance ratios are found in the following manner. First, the total variation as represented by the mean site index of 66 feet and the range of 47 feet to 88 feet is expressed in terms of variance by conventional methods of calculation. The total variation is then divided into that part which is explained by the site factor and remainder, which is that part unexplained by the site factor. The ratio of these two parts is the variance ratio. A large variance ratio indicates that the site factor explains a large part of the site-index variation among stands. Variance ratios that are statistically significant are marked with asterisks in table 1.

The means or regressions for the five factors that were significantly associated with variations in shipmast locust site index are shown in column 4. While these relationships were significant, they were somewhat deceptive when considered without regard to one another. Because of interrelationships among them, several appeared to be more important than they actually were, while others that actually were important did not show a significant variance ratio when considered individually. Attention was therefore given to various combinations of site factors. As a result of this examination, the site classification shown in figure 2 was devised. This classification accounted for all

the significant relationships between growth rate and the site factors listed in table 1.

Figure 2 is used in the following manner. First, the site is classified into either of two topography groups: hillsides or flat terrain. By hillside stands is meant stands located on sloping land where a drainage area lies above the site. Stands in this group were fed by supplementary drainage water as evidenced by the frequent occurrence of springs and seep spots. By stands on flat terrain is meant stands located on either level, undulating, or sloping land where no drainage area lies above the site. In general, stands on hillsides showed markedly better growth than those on flat terrain.

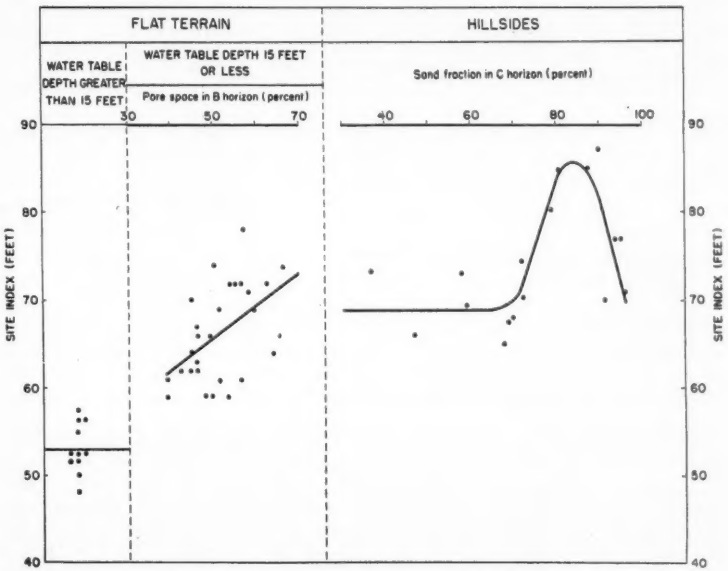

FIGURE 2.—Site classification for shipmast locust on Long Island. The plotted points are data from 56 measured stands.

Hillside stands are further classified according to the percentage of sand in the C horizon. Growth rate was more rapid on sites where the sand concentration of the C horizon was between 70 and 90 percent than on sites where the soil contained a greater proportion of finer particles or where the soil was extremely sandy.

Stands on flat terrain are next classified into two groups according to depth to a water table. On sites where the water table was deeper than 15 feet (16 to 135 feet in these samples), the average site index was 53.6 feet. On flat terrain where the water table was 15 feet or less (4 to 15 feet in these samples) the growth rate was more rapid. Among these latter stands the site index varied according to the pore space in the B horizon, the growth rate being better on the more porous soils.

The site classification of figure 2 was more effective for explaining variations in growth rate than any other set of factors that could be compounded from the 39 individual factors. The extent to which growth rate variations were accounted for is shown by the analysis of variance of table 2. The total variation, represented by a sum of squares of 4,596, is segregated into one part, 902, not accounted for by the classification. The variance of this part is 19.2. The remainder of the sum of squares, amounting to 3,694, is the part explained by the classification. Thus, the classification accounts for 3694/4596, or roughly 80 percent of the total variation among stands. The relative importance of the 4 factors used in the classification is shown by the further analysis of the sum of squares. Referring to the classification of figure 2, the variance accounted for by topography was 1,571, by water table depth 1,341, by texture 114, and by pore space 213. All these values are highly significant in comparison with 19.2, the remainder variance.

TABLE 2.—*Analysis of variance of site index for 56 stands classified according to figure 3*

Item	Degrees of freedom	Sum of squares	Variance
Variation explained by each factor of the classification:			
Topography	1	1,571	1,571*
Water table depth	1	1,341	1,341*
Texture	5	569	114*
Pore space	1	213	213*
Variation unexplained by the classification	47	902	19.2
Total	55	4,596	

*Highly significant in comparison with unexplained variance.

This classification accounted also for the significance of the other factors listed in table 1, i. e., slope, phosphate in C horizon, soil type, and aspect. This was determined by subjecting the remainder variance of 19.2 to further examination for the purpose of determining whether the variations in growth rate which it represents were explained by relationships with any of these five factors. No significant reduction below 19.2 was obtainable, indicating that when the site classification of figure 2 was used, the other site factors were without significance. The expected site index for each stand was determined from figure 2. The deviations of these expected site indexes from the actual site indexes were calculated and compared with the remaining site factors. None of these factors accounted for the deviations. Apparently, factors not included in this study, such as stand history, caused these deviations.

It is worth while mentioning that the site factors of figure 2 explained almost entirely the variations in growth rate associated with soil series. There were four major soil series (*11*) among the stands, and, as indicated in table 1, their associated growth rates differed significantly. However, these differences in growth rate were accounted for largely by the differences between the soil types in topography, water-table depths, texture, and pore space.

VEGETATION AS AN INDEX OF GROWTH RATE

The natural vegetation in the area where shipmast locust occurs on Long Island may be divided into two main types. In the more mesophytic forests, located principally on the moraines near the north shore, typical moisture-loving species, such as walnut, beech, and yellow poplar, occur. The less mesophytic vegetation is found principally on the relatively dry outwash plains south of the moraines. Typical species of this forest are white oak, chestnut oak, pin oak; and on the driest sites, pitch pine and scrub oak.

Study of the flora in the shipmast locust stands indicated that the presence of several tree and ground-cover species was associated with the growth rate of shipmast locust. On sites where yellow poplar (*Liriodendron tulipifera* L.) and celandine (*Chelidonium majus* L.) were present, shipmast locust made its best growth. The average site index for shipmast locust on the four sites in which yellow poplar was found was 78 feet, and on the four sites in which celandine was found, 74 feet. These site indexes were well above the average of 66 feet for all the stands studied. Of the seven stands in which these two species occurred, six were classed as hillside stands and one as flat terrain with a water table less than 15 feet deep. This classification indicated that favorable soil moisture conditions existed in these sites.

Other species which appear to have some value for indexing growth rate of shipmast locust on Long Island were sassafras (*Sassafras variifolium* (Salisb.) Ktze.) and white oak (*Quercus alba* L.). These species occurred in stands where the growth of shipmast locust ranged from average to poor. The average site index of the 6 stands in which sassafras occurred was 63 feet, and that of the 6 stands in which white oak occurred, 59 feet. Most of the other 80 associated species of trees, shrubs, and ground-cover plants were either in insufficient quantity or were distributed over too wide a range to indicate any relationship with the growth of shipmast locust.

Since the species mentioned have generally recognized site requirements, their presence should be useful as an approximate check to supplement and confirm site evaluations made by means of the topographic and edaphic classification shown in figure 2.

RELATION OF SITE TO VIGOR OF SHIPMAST LOCUST

A striking feature of the shipmast locust stands on Long Island is the frequency of partly or entirely dead crowns. The trees grow well for a number of years, and then the upper part of the crown dies, sometimes as much as a quarter or a half of its length. The size of the trees at the time top decadence starts varies with the site.

Data on crown vigor in relation to site were obtained in 76 shipmast locust stands. On the basis of the largest trees, each stand was classified as to vigor by the following standards:

Vigor class	*Condition of crown*
1	Tops of all trees healthy.
2	Tops of some or all trees partly dead.
3	Tops of all trees dead.

The site index for each stand was determined from measurements on smaller trees in which decadence had not yet set in. In 52 of the

stands, undergrowth of competing native trees and shrubs was present. The remaining 24 stands were clear of competing trees and shrubs, although a grass and herb ground cover was present in all stands.

The data (table 3) indicate that the more vigorous stands were associated with higher site index. On sites of similar quality, as shown by stands in the same site-index class, vigor declined as the trees became taller. The prevalence of crown decadence in shipmast locust on Long Island is indicated by approximately 60 percent of the stands having partly dead crowns.

TABLE 3.—*Average height of the tallest trees and site index for 76 stands classified according to crown vigor*

Site index	Vigor class 1 (15 stands)		Vigor class 2 (46 stands)		Vigor class 3 (15 stands)	
	Frequency	Average maximum height	Frequency	Average maximum height	Frequency	Average maximum height
	Number	*Feet*	*Number*	*Feet*	*Number*	*Feet*
80–89	4	78	2	96		
70–79	7	47	15	69	2	82
60–69	4	40	18	60	4	76
50–59			11	43	5	49
40–49					2	52
30–39					2	32
Average site index	74		66		56	

As a result of this examination, figure 3 was constructed. It shows for Long Island the maximum height to which shipmast locust grows and the height at which the various degrees of decadence occur for a range of site indexes. To use this chart as a guide in selecting sites or for cultural operations, the site index is first estimated, either by judging the growth-producing qualities of the site before planting or by measuring growth rate of the shipmast locust trees a few years after planting. For any site index, the approximate maximum height attainable is shown by line D, and the vigor at different heights by lines A, B, and C. On poor sites, for example, where the site index was 40, no stands over approximately 50 feet were observed, and stands above 30 feet were completely dead in the top. Hence, complete decadence occurred on such sites before the trees were large enough to produce a good yield of fence posts. On areas where the site index was 80, the trees reached a maximum height of over 100 feet and a height of 75 feet or more before decadence began (line A). This size is great enough for the production not only of fence posts, but also of barn sills, planks, piling, or railroad ties.

One feature of the chart that requires a further word of explanation is the use of two division lines (lines B and C) to separate vigor classes 2 and 3. It was observed that in stands where undergrowth of shrubs and other trees was removed, the shipmast locust trees reached a greater height before becoming completely decadent than in stands of equal site index where undergrowth was present. This was shown by comparing different stands, as well as parts of the same stand where the owners had removed the undergrowth in one part and not in the

other. These observations indicate that shipmast locust on poor sites can be grown to a greater height before becoming severely decadent if the stand is cleared of undergrowth or if undergrowth is kept out of the plantation by appropriate cultural treatments.

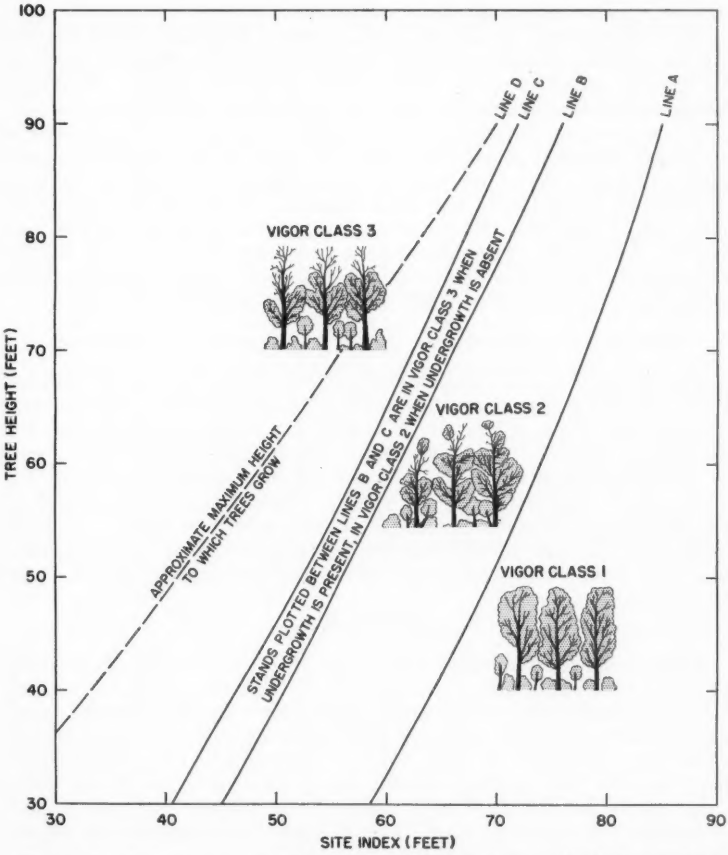

FIGURE 3.—Crown vigor of shipmast locust in relation to site index and tree height: Line *A*, start of crown decadence; line *B*, start of severe crown decadence in stands where undergrowth is present; line *C*, start of severe crown decadence in stands where undergrowth is removed; line D, maximum tree height.

The cause of decadence in shipmast locust is not definitely known. Examination of dead branches in the crowns showed the presence of borer holes, indicating that insects, probably *Cyllene robiniae* (Forst.), were one of the direct factors involved. Cope's observations (*4*) indicate that low winter temperature is a cause of crown decadence in shipmast locust. Whatever the direct cause of death, it is apparent from the data obtained in this study that predisposing factors are the

quality of the site, the height of the trees, and the undergrowth in the stand. As already shown, site quality in shipmast locust on Long Island is closely related to soil moisture supply. Undergrowth is important presumably because it also affects the amount of soil moisture available to the trees. It thus appears that a deficit in water supply may be the reason for top decadence.

A point to recall in this connection is that shipmast locust is not a native of Long Island, and therefore the crown development may not be entirely adapted to the existing soil moisture supply. This may be the reason why decadence increases as the trees grow in height.

An interesting illustration of the relation between tree height and vigor was observed in "topped" trees. Because of the unsightly appearance which decadent trees present, it has become common practice on landscaped areas to cut the dead tops out of such trees. Once the trees are topped, the lower part of the crown continues to live in a healthy condition. This observation suggested that the trees became decadent because they had reached a greater height than could be sustained permanently by their roots under the existing conditions.

GROWTH RATE OF SHIPMAST LOCUST AND COMMON BLACK LOCUST

The growth rate of shipmast locust in comparison with common black locust was determined from stand measurements over the same general area of Long Island. A total of 397 shipmast locust trees and 96 black locust trees were measured. The results are plotted in figure 4. Common black locust grew faster, on the average, than shipmast locust up to approximately 50 years. Thereafter, height growth of common black locust declined, while shipmast locust tended to continue its growth. The tallest shipmast locust trees measured were somewhat over 100 feet, which far surpassed the tallest common black locust in this area.

DISCUSSION

Since Long Island represents a specific combination of climatic, physiographic, and soil conditions (*11*, *17*) that do not exist in exactly the same degree and relationship anywhere else, the specific values given above must be considered applicable only to the area studied. However, the practical importance of this study lies in the aid it may furnish for judging growth rate possibilities in places where shipmast locust is introduced outside Long Island. In this respect, therefore, the implications of these findings have significance beyond the exact numerical relationships presented.

Growth rate of established shipmast locust on Long Island appears to be influenced mainly by soil moisture supply and soil aeration. Two of the factors, namely, topography and depth of the water table are fundamentally indicative of moisture supply. The best growth was obtained in stands having favorable conditions of moisture supply and loose-structured soil. Poor growth occurred on flat or rolling terrain where the water table was deeper than 15 feet, even when the soil was not compact. The importance of water supply was shown also by the recognized requirements of the vegetation found to be indicative of site quality for shipmast locust. Some of the good sites for shipmast locust were vegetated naturally by yellow poplar and

celandine. The poor sites were represented for the most part by the flat so-called "sand plain" of Long Island, where white oak and sassafras were common. This type of site was usually associated with extreme decadence of shipmast locust, as indicated by dead tops in the crowns and failure of the trees to grow as tall as on other sites.

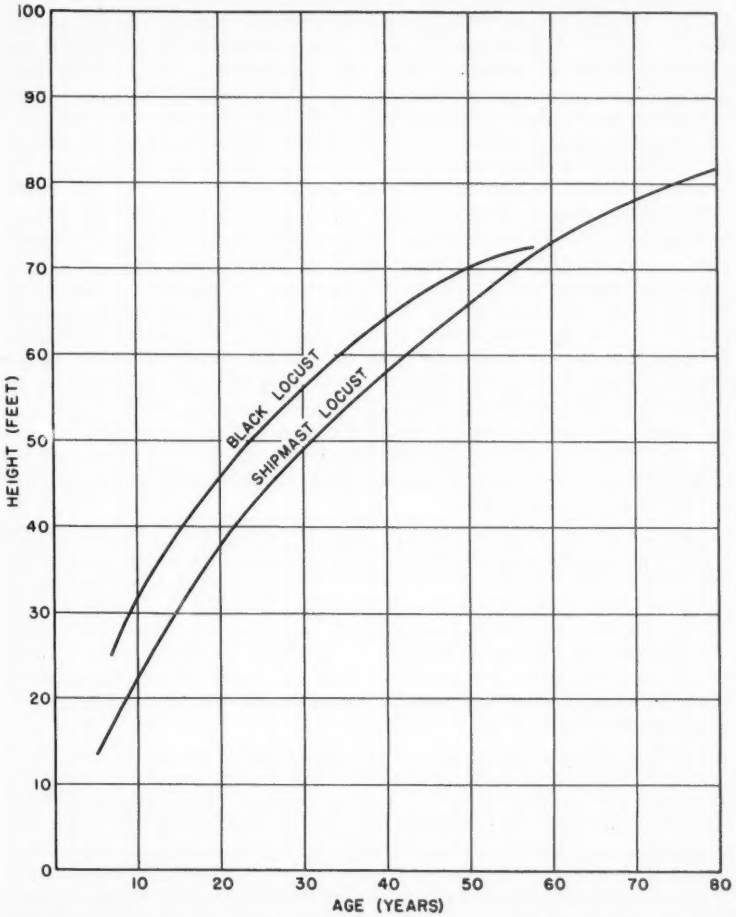

FIGURE 4.—Height growth of shipmast locust and common black locust on Long Island.

The trees measured in this study were in old established stands or sprout stands that came up voluntarily. These sites were all suitable for establishment and early survival. However, in selecting areas for planting, a wider range of conditions would be met. Some of these would be unsuitable even for initial establishment, while others would

be suitable for establishment but not for survival to maturity. Insofar as the site factors influencing establishment and early survival are the same as those that influence growth rate in mature trees, the general results of this study probably have some bearing on early growth in plantations.

Further light on this point is found in studies by Auten (*1*) and Grant (*6*) on the growth of common black locust. In these two studies, internal drainage of the soil was found to be the most significant factor in selecting sites for plantations. Soils with poor internal drainage were undesirable. In the present study, none of the stands were on poorly drained soils. The heaviest soil contained only 28 percent clay. Further, where moisture conditions were favorable, good internal drainage affected growth rate, as indicated by the relationships shown in figure 2.

Since the growth of shipmast locust was good where yellow poplar was present, a study by Auten (*2*) on the factors influencing growth rate of second-growth yellow poplar is noteworthy. He found topography and soil texture to be important. These are the same two factors that proved to be effective for indexing shipmast locust growth rate in this study. However, Auten's further division of topography according to aspect, exposure, and position did not hold for shipmast locust. The classification shown in figure 2 did not predict growth rate exactly, but variations from the predicted values were not related to aspect, exposure, position, or any other of the factors listed in table 1. This discrepancy may be due to the generally humid conditions on Long Island, or else shipmast locust is not as sensitive as yellow poplar to these location factors.

The better growth on hillsides having a drainage area above the stand was attributable apparently to better soil moisture conditions. This was indicated by the frequency of springs and seep spots on many of these sites. On some of the sites where surface water outlets were not observed, wells were present, and the water in these wells stood but a few feet below the ground surface. The manner in which a hillside topography brings about these soil moisture conditions, even when bedrock is absent, is shown by the studies of Norling (*12*) and Warington (*20*).

In view of the tendency to early top decadence on poor sites, it would be well for planters to consider both figures 2 and 3 in selecting sites. If we arbitrarily set 50 feet as the minimum height of trees for economic fence-post production, and 75 feet as the minimum for production of larger timbers, reference to figure 3 indicates site-index requirements of approximately 60 and 75, respectively. Now turning to figure 2, we see that, for Long Island conditions, sites located where the water table was deeper than 15 feet would generally be unsuitable for fence-post production, because such sites have indexes usually below 60. For production of large timbers, only hillsides with a moderately high sand concentration in the C horizon would be promising sites.

SUMMARY

Based on site and growth measurements in 56 stands, a method of classifying site productivity for shipmast locust on Long Island, N. Y., was developed in which the following four factors were used: (1) Topography; (2) depth of water table; (3) soil texture; (4) soil pore space. This classification accounted for approximately 80 percent of the total variation in growth rate among the 56 stands. When these 4 factors were used, none of the other 35 site factors measured in this study had any apparent influence on growth rate.

Best growth occurred on hillsides and on flat terrain where the water table was between 4 and 15 feet deep, provided the soil was well aerated but not excessively sandy. Flat terrain where the water table was deeper than 15 feet, waterlogged soil, and heavy, poorly aerated soil were relatively unfavorable. The most favorable sites were those capable of supporting native growth of yellow poplar and celandine.

The height to which shipmast locust grew on Long Island varied with the quality of the site. On the best sites, shipmast locust attained a height of 100 feet or more, but on poor sites, the upper part of the crown died and height growth ceased. A chart is given which shows the maximum height to which shipmast locust grows and the heights at which various degrees of decadence occur for a range of site indexes on Long Island. Stands kept clear of competing tall vegetation reached a greater height before the trees became decadent. The limitation that site imposes on the commercial products attainable can be taken into consideration in selecting planting areas through the use of this chart.

Comparison of 397 shipmast locust and 96 common black locust trees located within the same general area indicated that the former grows more slowly in height until approximately 50 years, but thereafter more rapidly.

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ORIGIN OF THE SEED COATS IN GUAYULE[1]

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INTRODUCTION

The delayed germination of seed of guayule (*Parthenium argentatum* A. Gray) is attributable to two important factors, namely, an embryo dormancy of approximately 2 months' duration and the interfering action of a seed coat (*2*).[2] After the period of embryo dormancy satisfactory germination has been obtained by treating the seed with sodium hypochlorite or other oxidizing agents such as hydrogen peroxide or nitric acid. Benedict and Robinson (*2*) found that it is the inner seed coat which delays germination, and when this is removed chemical treatment is unnecessary. They found further that puncturing the inner seed coat with a needle or placing the seed with an intact inner seed coat in a high partial pressure of oxygen would induce germination. These results indicate that sufficient oxygen for germination does not ordinarily reach the embryo. Whether the inner seed coat consumes oxygen or is nearly impermeable to it is not known.

Borthwick and Robbins (*3, pp. 284–285*) stated that for lettuce, another composite, "the oxygen requirements increase rapidly with an increase in temperature, such that an adequate supply fails to diffuse through the membrane at the higher temperatures." Thus, at 30° C. or above untreated lettuce seed fails to germinate in air unless the seed coat is removed. Davis (*4*) presented evidence to indicate that the seed coats of lettuce become less permeable to gases at higher temperatures. The findings of Thornton (*10*) do not indicate a simple impermeability of the membrane of lettuce seed, since a high partial pressure of carbon dioxide induced germination at temperatures which ordinarily inhibit it. Borthwick and Robbins (*3*) found that in lettuce the seed coat immediately investing the embryo is made up of one or two layers of thick-walled endosperm cells and the inner cell wall of the inner layer of cells in the integument. They were under the impression that it was this integumentary membrane which impeded oxygen diffusion to the embryo.

The present study of the origin of the seed coats in guayule was undertaken as a preliminary step in a further investigation of the interfering action of the seed coat on germination.

MATERIALS AND METHODS

Eighteen guayule plants of strain 593 in 8-inch pots were transferred from outdoor tables to the greenhouse on February 23. Within 3 weeks the plants were flowering. Heads in various stages of development were killed and fixed in Randolph's modification of Navashin's

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[2] Italic numbers in parentheses refer to Literature Cited, p. 334.

fluid (*7*) for paraffin sectioning. Dehydration was done with tertiary butyl alcohol. Sections were stained with safranine (*5*) and gentian violet dissolved in clove oil. Buds in young stages were fixed without dissection, but only the ray flowers were used in older stages. The mature seed was soaked in water for a few hours before dissection and fixation.

ANATOMY

The flowers of guayule were briefly described by Artschwager (*1*), and accounts of the morphology of reproduction were given by Kirkwood (*9*) and Esau (*6*). The present investigation was particularly concerned with the development of the anatropous ovule and the destiny of its cell layers.

A single massive integument forms in guayule, and at the stage when the megaspore mother cell appears the integument has not yet completely enveloped the uniseriate nucellus (fig. 1, *A*). In many respects the ovule is similar to that of lettuce (*8*). The nucellus in guayule, however, is fused to the integument along most of its length; this condition is in marked contrast to that in lettuce, in which the nucellus is free from the integument almost to the chalazal end (*8*).

As in lettuce, the integument of guayule produces a nutritive jacket; this forms around all but the micropylar end of the embryo sac (fig. 1, *B* and *C*). It is derived from the inner cell layer of the integument and is a prominent feature during the development of the ovule. The nucellus, on the other hand, becomes crushed except on occasions such as those when a second embryo sac begins to form in one or more of its cells. Most of the early cell divisions in the nutritive jacket are anticlinal, but in later stages the jacket may become two cells or more thick. It is always thickest near the micropylar end of the ovule (fig. 2, *A* and *B*); it does not completely enclose this end, however, as it does the chalazal one.

The endosperm typically becomes cellular at an early stage in the development of the embryo (fig. 1, *C*), and soon it is composed of large and small cells. The large ones occupy a central position, whereas the small ones line the embryo sac. The endosperm tissue is most prominent at the micropylar end of the ovule, where it is persistent (fig. 2), but except for one or two layers of cells it is obliterated elsewhere as the embryo enlarges (fig. 3).

Destruction of intermediate layers of integumentary cells commences even before the embryo sac is mature; it progresses most rapidly at the chalazal end and permits most of the enlargement of the embryo sac to take place in this direction. Except for the outer cell layer of the integument and the vascular bundle, this ovuliferous structure is destroyed during the maturation of the seed (fig. 3). The last of these cell layers to be destroyed are the subepidermal ones and the nutritive jacket (fig. 3). The latter finally loses its densely protoplasmic appearance, a very characteristic feature retained during the development of the ovule and prior to maturation of the seed. During this final stage (fig. 3, *B*) the endosperm cells show signs of wall thickening at the outer corners.

The integumentary membrane of lettuce (*3*) is similar in appearance to a membrane found in guayule seed (figs. 2, *B* and *C*; 3, *C*). However, during the development of the guayule seed it was found that in

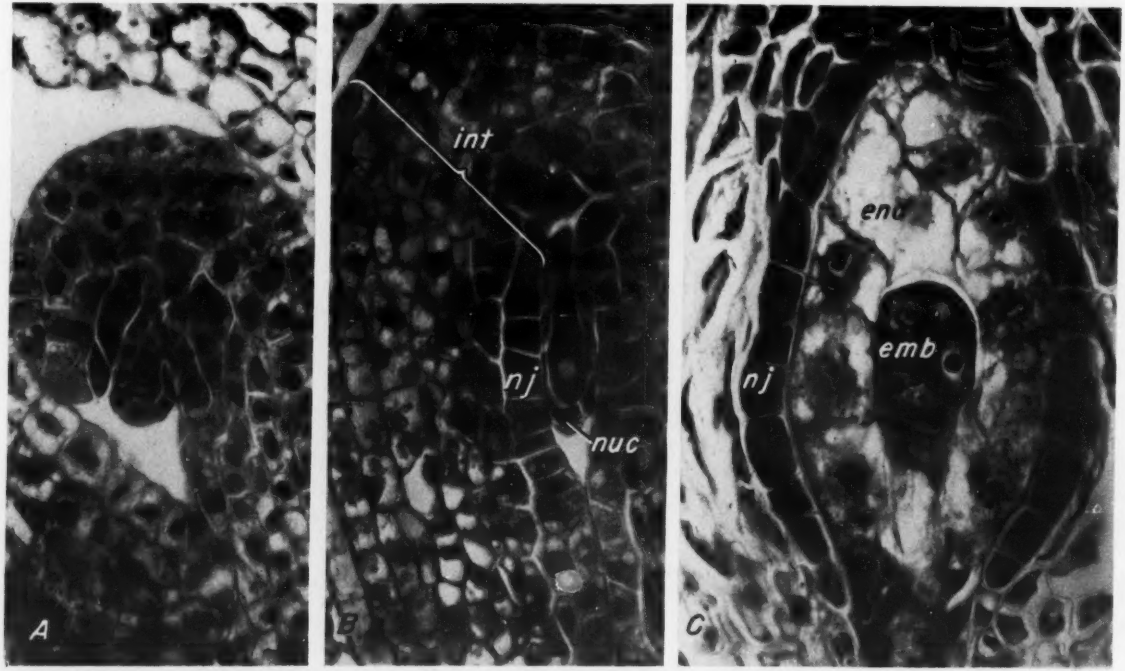

FIGURE 1.—Sections of guayule ovules: *A*, Young ovule; *B*, slightly older ovule showing origin of nutritive jacket and collapse of part of nucellus; *C*, ovule with young embryo. All × 440. *emb*, Embryo; *end*, endosperm; *int*, integument; *nj*, nutritive jacket; *nuc*, nucellus.

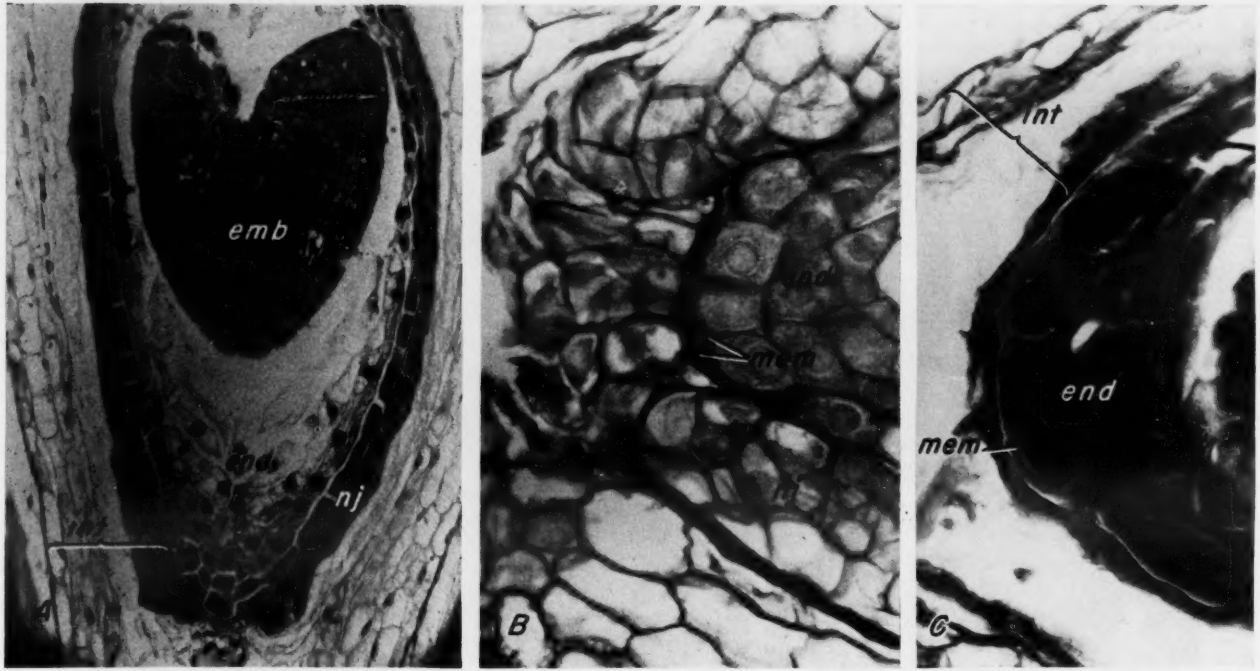

FIGURE 2.—Sections of guayule ovule and seeds. *A*, Ovule with immature embryo; × 190. *B*, Micropylar end of immature seed, showing formation of membrane between endosperm cells; × 440. *C*, Micropylar end of mature seed; × 490. *emb*, Embryo; *end*, endosperm; *int*, integument; *mem*, membrane; *nj*, nutritive jacket.

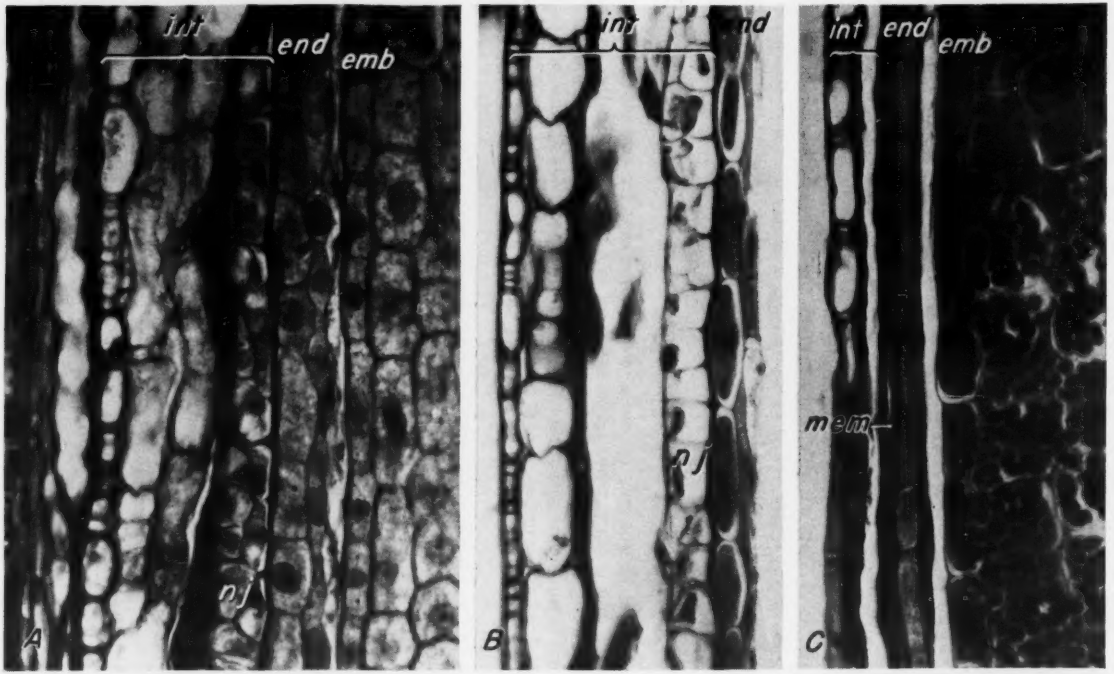

FIGURE 3.—Sections of guayule seeds: *A* and *B*, Immature seeds; *C*, mature seed. All × 440. *emb*, Embryo; *end*, endosperm; *int*, integument; *mem*, membrane; *nj*, nutritive jacket.

the micropylar region where the nutritive jacket did not enclose all the endosperm cells a thick wall-like material was formed; this occupied positions even between endosperm cells (fig. 2, *B*, *mem*). In view of the occurrence of this membrane where integumentary cells are lacking and also between certain endosperm cells, it appears that a so-called integumentary membrane as found in guayule, and perhaps in other composites, may in reality be a wall material excreted by the endosperm cells.

At the present time it has not been determined whether the dormancy in guayule seed is due to the membrane enclosing the endosperm or to the walls or living protoplasts of the endosperm cells.

After the pericarp of a mature guayule achene has been removed, the thin, white, soft, outer seed coat, which readily peels off, is the outer cell layer of the former integument. It is one cell thick except in the region where the vascular bundle adheres, and the cells are dead. The inner seed coat, which closely invests the embryo, is also thin and white but very tough. It is composed of a thick wall-like material and one or two cell layers of living endosperm cells. At the micropylar end of the seed the inner coat is somewhat thicker, because of the presence of endosperm tissue several cells in thickness.

SUMMARY

The achene of guayule contains a seed enclosed by two seed coats. The outer one is thin, white, and soft and is a single cell in thickness except where the vascular bundle occurs. It originates from the outer cell layer of the integument. The inner seed coat is thin, white, and tough and is composed of a membrane and one or two cell layers of living, thick-walled endosperm cells. At the micropylar end this seed coat is usually several cells thick. The membrane appears to be formed, at least in the micropylar region, by excretion of material from the endosperm cells.

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